A NEW METHOD FOR THE STUDY OF CHROMOSOME ABERRATIONS AND THE PLOTTING OF CHROMOSOME MAPS IN DROSOPHILA MELANOGASTER

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For many years *Drosophila melanogaster* has been a classical object for genetic studies but, due to the small size of the chromosomes and to the fact that we have been able to make little headway in the study of these elements in meiosis, it has been necessary to turn to less favorable material genetically, in order to study some of the questions which have arisen with the progress of cytogenetics. The past fall the writer began a study of the salivary glands in larvae of this fly with the hope that it would be possible to recognize the same chromosome in any cell, and in this way to get more accurate measurements of translocated pieces and, in general, to gain some insight as to the nature of dislocations of chromatin. It has been found that the chromosomes in the salivary glands have a definite and, within limits to be described, a constant morphology which enables one to recognize the same element in any cell. The elements are so distinct and constant in their morphology that we can follow a translocated segment to its new site, or if we are dealing with a mutual translocation, can determine just how much of each chromosome is involved in the exchange. Needless to say, this places in our hands a method of studying translocations and of plotting cytological maps which is a vast improvement over the study of metaphase plates heretofore used. But what is of far greater importance is the discovery, wholly unexpected, that in the salivary glands of old larvae the chromosomes may undergo somatic synapsis. As a result, homologues pair line for line, band for band, and unite into one apparent element. If, however, one of the homologues has an inverted section, we get typical inversion figures, such as may be seen in the meiosis of corn, and by the bands and other landmarks we can tell, cytologically, just where the inversion occurred. If one chromosome has been deleted, it pairs with its homologue except at the region of the deficiency; here the normal chromosome buckles and by comparing the lines of the two elements we can tell just how much is missing from the deleted element. This gives us a new and relatively exact method for the study of inversions and deficiencies and enables us to make a direct attack upon many of the problems associated with these aberrations.

As new as the facts reported above may appear, the morphological observations upon which they are founded (unknown to the writer at the

time he reached the above conclusions) have been recorded in the literature for many years, for some of the other Diptera. He, therefore, can only lay claim to having shown, by a study of inversions, the intimate character of the union of homologues and to the rather obvious application of the findings to a study of present day problems in cytogenetics. The most recent paper on this general subject is that of HEITZ and BAUER (1933) dealing with *Bibio hortulanus*, in which they report that in the Malpighian vessels the haploid number of chromosomes may be found due to the union of homologues. They maintain that the different elements in the nucleus can be identified in any cell by such differences in morphology as length, method of terminalizations, association with plasmosomes, etc. There is nothing in this paper nor in the earlier literature reviewed by them, which would indicate that the association of homologues, so often seen, was anything more than a close apposition of elements.

The purpose of this paper is to give some of the evidence upon which the conclusions presented in the first paragraphs are founded, to point out the usefulness of the material for cytogenetic studies, and to give some of the outstanding conclusions which have been reached. It is planned to publish detailed studies dealing in turn with the separate elements of the complex, and with the general question of chromosome structure. A paper dealing with the X chromosome is now in press in GENETICS.

At this place the writer wishes to express his thanks to his colleague, Mr. WILSON STONE, whose intimate knowledge of the genetic make-up of the stocks in this laboratory has been an invaluable aid in making this study.

The method employed for the preparation of slides is simply to dissect out the salivary glands of old larvae about ready to pupate, place these on a slide, add aceto-carmine, cover with a cover glass and stain for a few minutes. Then crush the glands by pressing on the cover glass with a needle, remove the excess of stain with filter paper, seal with vaseline and study under blue-green light. The slides show up best a day or two after preparation.

When one has prepared a slide from an old female larva of normal stock, under low power a number of nuclei will usually be seen which are conspicuous because the contained elements are large and deeply staining; these should be selected for study. In figure 1 we have a drawing of such a cell in which the nuclear wall has been broken and the contained elements spread apart so that we can follow each from one end to the other. There are six conspicuous elements in this nucleus. One is small, roughly two and a half times as long as its diameter; this represents the two fourth chromosomes so closely apposed that we get no hint of its compound nature. Of the five long elements, one represents the two X chromosomes,

which like the fourth chromosomes, are so closely joined that they appear as one. The four remaining elements are the left and right arms of the second and third chromosomes, respectively. All elements are labelled in figure 1. The identification of the several elements was made possible by a study of appropriate translocations and inversions, as will be described, in part, in the following pages.

All six of the elements are normally embedded at the spindle fiber end in a chromatic (with Feulgen's stain) coagulum or mesh work. In most preparations there is no indication of which arms belong together (in the cases of the second and third chromosomes) but occasionally, in crushing the nucleus, the elements are separated from the mesh work and in this event it is not unusual for two to remain attached together. In this way I came to associate what have proved to be the two arms of the second and third chromosomes, respectively, but I could not be certain of the identification until I had analyzed such stocks as Curly, which has inversions in either arm of the second, and various translocations involving both second, third and the fourth chromosomes. With the help of these stocks it has been possible not only to determine which arms belong together, but which are the so-called genetic right and left arms respectively.

A close study of any element such as II L in figure 1, that is, the genetic left arm of the second chromosome, shows the more or less achromatic matrix or substratum which has the general proportions of an ordinary earth worm. Lying either on the surface or just under the surface of this matrix we observe what appear to be chromatic bands (often described as discs). The cylindrical matrix shows a fairly uniform diameter throughout its length, but certain elements show local differentiations which within limits may be constant morphological features. In the case of the left arm of the second chromosome there are four such "vesicular areas." The matrix, at least after a few minutes fixation in aceto-carmine, is quite tough so that it may be stretched to two or three times its normal length with a consequent decrease in its diameter. The bands which strike one's eye, on the superficial examination of any preparation, are really made up of a number of lines (frequently two). These lines may be classified, in general, as thick and densely chromatic, thin and sharply chromatic, diffuse or fuzzy, discontinuously chromatic giving a series of dots or dashes, and finally, very thin in diameter and more or less faintly staining. One or more of these different types of lines go to make up a "band" in a relatively contracted area of any chromosome. It is exceedingly important to realize this fact because, when the matrix is stretched or otherwise distorted, the lines of a prominent band may be so far separated that the existence of a "band" is no longer obvious.

As we study the same chromosome in different preparations, in normal

stocks, we may get superficially quite different images. This may be due to differences in fixation and in the degree of staining, but principally to the degree to which the chromosome or arm is contracted, or stretched, or mashed. A freshly stained preparation shows the matrix as achromatic and the lines of the bands as lightly stained. But as the preparation gets older the lines stain darker red and the matrix, especially close to the lines, may be tinted with the dye. In old preparations one must study the lines of a "band" with care because when the matrix is stained, we may see a band, on superficial examination, which really doesn't exist. At the outset, one must be careful not to be misled by the presence of broken chromosomes, for experience has shown that certain elements are liable to break at certain definite weak points.

Attention should be called to the fact that there is a tendency, on the part of the observer, to focus on the matrix at the point of its greatest diameter. Since the matrix is appreciably thick, we do not see the lines on its upper surface clearly, and there is a shadow of the lines on the lower side. The net effect is a hazy band, an effect which is heightened by the somewhat oblique course which the lines often show. In focusing sharply on either the upper or lower surface of the matrix the true situation will be revealed and the vague band will disappear.

With the above points in mind we may now study several of the individual chromosomes or arms. The most easily identified element in the nucleus is the genetic left arm of the second chromosome. This is labelled in figure 1. Drawings of this same element, taken from other cells, are given in figures 2 to 7, the latter being the inversion figure obtained when a female heterozygous for Curly is studied. Using figure 2 as typical, and beginning at the distal end (to the left in figure 2) we may conveniently divide this arm into several sectors which have very definite boundaries. Sector *a* begins with the terminal bulb and runs through the heavy band bounding the first vesicular area. Sector *b* begins with the just named vesicular area and runs through the heavy band bounding the second vesicular area. Likewise sector *c* begins with this vesicular area and runs to the midpoint of a very prominent constriction. Sector *d* comprises the rest of this chromosome arm. All of these areas are indicated on the drawing with appropriate labels.

Returning to sector *a*, and beginning with the terminal bulb, we note towards its base, first a sharply staining line followed by two thin lines, and second a very heavy line (usually appearing as double) labelled a_1. (Ultimately, it is hoped to replace this nomenclature by the symbols of the genes which lie at these points along the chromosome as has been done in the case of the X chromosome.) This is followed by a band less heavy (a_2) and then a very heavy band, a_3 comprising at least 3 heavy

lines which is as thick as a_1 and a_2 put together. About three diameters of the matrix farther along we have a very heavily banded area (a_4), often showing four heavy lines, and finally the limiting band of the area (a_5). Figures 3 to 6 illustrate the appearance of this terminal sector taken from

FIGURE 1.—This camera lucida drawing, taken from an aceto-carmine preparation, shows the chromosomes found in a salivary gland after the nuclear wall had been ruptured by pressing on the cover glass. In this case, somatic synapsis has been completed. The various elements are labelled, II L being the genetic left arm of the second chromosome.

other preparations. In a similar manner, the various other areas of this chromosome arm may be identified. Figure 7 is the figure obtained when a female larva heterozygous for Curly is studied. It has long been known

genetically that this arm is inverted in Curly. Again we can follow the regional differentiations with ease. The inversion has obviously occurred between bands a_3 and a_4 on the distal side of the arm and since the elements are paired throughout sectors *b*, *c*, and a part of *d*, it is clear that the other limit of the inverted area is about a third of the length of sector *d*. As a matter of fact, a close study of a number of figures has shown that the proximal limit of the inversion is between two specific banded areas. Needless to say, if we knew genetically the limiting gene loci of this inversion we could plot the position of such loci with great exactness.

In comparing the figures of this left arm of the second chromosome in the drawings (figures 1, 2, 3, to 7) the reader will gain a fair idea of the constancy of the landmarks described as well as the variations in the superficial appearance of this element in different preparations. The sources of these variations has been discussed above.

In order to show the typical and undistorted structure of the X chromosome figure 8 (shown in two sections) has been prepared by combining a number of camera lucida drawings of this element. Thus each feature is an actual camera lucida sketch taken from a drawing which showed the given area in typical form. The approximate location of some of the gene loci already determined is shown by appropriate symbols. There are three features of the X which are useful in identifying this element. The first is the terminal bulb (to the left in the upper section of figure 8) taken together with the fact that the whole left hand half of the X shows no relatively deeply staining and broad banded areas. About the middle of the X in the region of *v* (vermilion) are two very conspicuous bands, which stain very deeply, the one to the left being about twice as broad as the one to the right. It may be noted that in my preparations there is some tendency for the X to break at the constriction to the right of the *m* (miniature) region. To the right of the *B* (Bar) region the X becomes heavily chromatic, the five deeply staining banded areas constituting, perhaps, the most conspicuous feature of the entire element. The spindle fiber end (to the right in the lower section) has a terminal bulb which carries a great deal of chromatin, but this area is subject to distortion due, presumably, to the fact that it is anchored here to the chromatic coagulum. By comparing figure 8 with the X of figure 1, the reader will be able to identify most of the prominent land marks. Parenthetically, it may be pointed out that the heavily chromatic part of the X lies well to the right of the Bar locus, and while, as this is written, I am not sure of the locus of carnation, we may say that this chromatic region is relatively poor in known active genes, if, indeed it carries any except the normal allelomorph of bobbed. Conversely, that region of the X most crowded with gene loci (the left

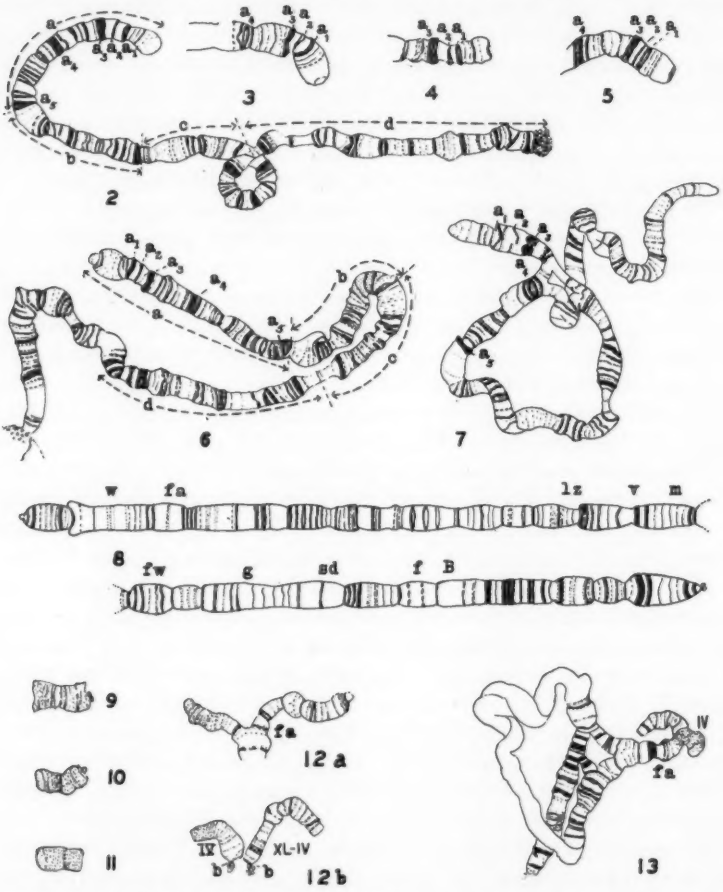
hand end) shows relatively very little chromatic material. These facts are obviously of great interest genetically as well as cytologically.

In figure 1, the characteristic appearance of the paired fourth chromosomes may be seen. It has a peculiar granular appearance unlike any of the other chromosomes and there are no heavy lines. Usually, one can identify three or four lines, a little more conspicuous than the rest, and the free end often appears a little denser than the spindle fiber area. The figures 9 to 11, three additional drawings of the paired fourth chromosomes are given. Two of these show, at the spindle fiber end one or two small, but very distinct, ring-like vesicles which probably represent the points of attachment of the chromosomes to the mitotic spindle. Similar structures are seen on the X and the second and third chromosome, there being typically two for each (paired) element.

The chromosome configurations shown in figures 12 and 13 are of especial interest since they illustrate both the *ClB* inversion of the X chromosome and a case of a mutual translocation between the X chromosome and the fourth. We will describe the latter first.

The stock involved is known in our laboratory as "Mottled 5," originally isolated by MULLER and studied genetically by BOLEN (1931). The genetic evidence in this case indicated that there had been a mutual translocation in which the gene for bent (located on the fourth) was linked with white eye and the other loci to the left of this locus on the X. Likewise, the other genes of the fourth were linked with the whole X chromosome to the right of white. Cytologically, the normal fourth and the XL-IV elements were not noticeably different in size in metaphase plates of oogonia. A study of the salivary gland cells is very illuminating as is shown in figure 12a and b, taken from the same nucleus. Figure 12a represents the left end of the paired X chromosomes. The fourth chromosome component lies to the left in this figure, the normal left hand end of the X to the right. Obviously, the original X broke near *fa* (facet) and part of the fourth chromosome took its place. Beyond this point the two X's are fused together. In figure 12b we have the normal fourth chromosome and XL-IV element. The reader will recognize the tip of the X lying to the right. The normal fourth and the XL-IV are joined, or, at least, lie close together at their spindle fiber ends. Both show one line in common labelled *b* (bent). The apparent spindle fiber attachment area of both elements is also seen. Since the volumes of the two elements (the normal fourth and the XL-IV) are not greatly different, it is clear why in metaphase plates of oogonia one could detect no difference in size between them. It should be noted that neither the IV-XR nor the XL-IV chromosomes show any scar at the point of break and translocation.

Figure 13 shows the points on the X chromosomes where the *ClB* inversion took place. This figure was taken from a female larva heterozygous for the "Mottled 5" X which is normal in genic sequence except for



FIGURES 2 TO 13.—The typical morphology of the left arm of the second chromosome is shown in figures 2 and 6. Figures 3 to 5 show the terminal segment taken from other cells. Figure 7 is the inversion figure found in the left arm of the second when the larva is heterozygous for Curly. Figure 8, made by combining camera lucida drawings, shows the typical structure of the X chromosome. The approximate location of a number of gene loci, as determined by a study of breaks, is indicated by the conventional symbols. Figure 9 and 11, illustrate the morphology of the paired fourth chromosomes. Figure 12a and b, taken from the same cell, illustrate a mutual translocation between the X and the fourth chromosome. Figure 13 is the inversion obtained, when a larva heterozygous for *ClB* is examined. In this case, the normal X carries a piece of the fourth chromosome on its left hand end.

the part involved in the translocation and a *ClB* chromosome. The *ClB* inversion occurred at the right hand end between the second and the fourth heavy bands of the series found in the proximal sixth of the X as described above, and we can see the fourth and fifth lines joined to the left hand end of the X at a point which, morphologically, is about three matrical diameters to the right of the lines separating white and facet. Except for the short distance where the arms diverge, the two elements are paired.

The description given for the left arm of the second, the X and the fourth will serve to illustrate the conditions in the salivary gland, and to bring out the value of the method for a study, especially of inversions, translocations, deletions and the plotting of chromosome maps. As this is written I have made no attempt to determine which are the genetic right and left arms of the third chromosome. One arm of the third, at the free terminal bud, often resembles the left hand end of the X, but the heavy bands immediately following, in the case of the third, should cause no one to be misled.

So far we have dealt with the elements of old female larvae. The condition encountered in the male is the same as for the female, except that the X chromosome does not stain deeply, and seems more apt to be distorted as if it were structurally much weaker. No sign of the Y is seen in the normal male, from which we might infer that it has fused with the X. This may, indeed, be the case. But in genetic setups where we should expect the Y free, I have been unable to identify it with certainty. In any event, it appears that special staining methods will be required to bring out the presence of this element.

DISCUSSION

Of the large number of questions which are raised by the present work, the writer proposes to take up here only a few points, primarily those concerning the validity of the method as a means of studying inversions, (for which it is a method *par excellence*), translocations and the position of gene loci.

The first point to be raised is, are the structures found in the nuclei of salivary glands chromosomes, and is there sufficient constancy in the elements of the salivary gland to enable us to identify the same chromosome or chromosome arm in different preparations? The evidence described in the foregoing pages has been presented, primarily, to show that this is true. Each chromosome (or arm) has a characteristic morphology expressed, for the most part, in the way the bands, or better, the constituent lines of the bands, are arranged and, to a lesser extent, in differentiations of the matrix. The arrangement of the bands is different in all the elements. During the process of pairing, which occurs in late larval stages homologues unite line for line, band for band. When one knows the charac-

teristic form of the normal elements, a translocated piece can be followed to its new position, or, in cases of mutual translocations, we have been able to determine the points at which each chromosome was broken and the extent of the exchange. If one homologue carries an inverted section, we can determine, cytologically, with relatively great exactness the points at which the inversion occurred.

All the above facts have been brought out and illustrated in the foregoing pages, and it seems unnecessary to enter into a lengthy discussion at this place. It should be emphasized, however, that while there is sufficient constancy in the sequential relation of the lines and differentiation of the matrix, to allow us to do the things enumerated, we must realize that the elements in the nucleus are labile and while the matrix shows considerable resistance to stresses such as mashing or stretching, these processes necessarily distort the superficial appearance of the chromosomes. Bands which, in the normal condition of the chromosome, are very useful criteria for identification through distortion of the matrix, may be pushed together locally, or so far separated that the banded effect is lost. Again, a mashed area of the matrix may stretch or break the lines running around the matrix, thus entirely altering the appearance of the region. But all these distortions are easily understood, once the labile nature of the chromosomes is appreciated and will give the experienced investigator no trouble. When we speak of constancy of chromosome morphology, we mean constancy in the relative position of the lines which go to make up the bands we ordinarily see.

The differentiations of the matrix are extremely varied. Many of the striking figures seen are undoubtedly artifacts and due to the technique employed. In other cases, however, vesicular areas are constantly seen at definite points along a given chromosome and these are of service in identification. In *D. melanogaster*, none of the elements show a constant association with the plasmosome, nor have I seen the elaborate terminalization figures described by HEITZ and BAUER. All of the elements have a more or less pronounced annulate or segmented form in that the matrix shows at irregular intervals slight local constrictions followed by bulges. These would seem to hint at the fundamentally compound nature of Drosophila chromosomes.

The question may now be raised, how do we know that the chromosomes of the salivary gland give us any proper conception of the structure of a "normal" chromosome? The answer is that since we have no direct cytological evidence concerning the structure of Drosophila chromosomes except that afforded by the salivary glands, the only way we can test the validity of the concept is to see how it fits in with such indirect evidence as we have. So far as cytology is concerned, it may be said at once that the

structure of the chromosomes, as revealed in the salivary gland nuclei, is in accord with the modern concept of the intimate structure of chromosomes obtained from other organisms, more especially from plants. The ideas of a matrix, a chromonemata carrying the gene string, and of local deposits of chromatic material around the gene string, have all found ample expression in cytological literature in recent years. Indeed, the picture we get from the salivary gland chromosomes allows us to reconcile the apparent conflict between the chromonemata and the chromomere concept of chromosome structure. For, if it is granted that chromatin is deposited locally along the spiral gene string, then we have a structural basis for the chromomeres, as they are commonly described in cytology. Since the writer proposes to take up the whole matter of chromosome structure in a later paper this phase of the subject may be concluded here with one remark, namely, that it seems to me inherently improbable that the fundamental structure of a chromosome will be materially altered by physiological activity.

So far as the genetic evidence is concerned, the concept of the structure of the X chromosome obtained from a study of the salivary gland is in very close agreement with the crossover map concept. In fact, with the possible exception of the locus for the normal allelomorph for facet, there is a close numerical agreement between the linear position of a gene locus on a schematic X chromosome 75.5 units long, and its crossover position (new map). On such a chromosome chart, the right hand limit of lozenge, for example, is about 35 units from the left hand end. That is to say, a break which, determined genetically, was between lozenge and vermilion, morphologically broke the chromosome some 35 units from the left end. While we do not know exactly where the break occurred genetically, we can say that lozenge lies to the left of the point of breakage. The crossover position of lozenge on the new map is 33.7. In a similar manner, we may say that the right hand limit of miniature, on our chromosome chart, is about 41 units from the left hand end. (The crossover value of miniature is 42.2.) And finally, Bar lies between the morphological limits of 58 and 62 units from the left hand end. Its position genetically is 62.2. From this it will be seen that the agreement between the new cytological map and the crossover map is very close.

The writer fully realizes that the position of Bar on the X chromosome in salivary glands is very different from the position of the element in metaphase plates of oogonia or nerve cells, as described by PAINTER (1931), STERN (1931), DOBZHANSKY (1932) and MULLER and PAINTER (1932). In metaphase plates the locus of Bar is near the middle of the chromosome while in the salivary gland chromosome it occupies its normal position judged by its crossover value. The question arises, what becomes of the

inert area of the X chromosome? It certainly does not show, as we might expect it to do, from a study of metaphase plates. This question is tied up with the problem of the Y chromosome and a discussion of the various possibilities will be given in my paper dealing with the X chromosome.

It has been clearly shown in the foregoing pages that in the nuclei of female larvae (and of males as well), homologous elements may join in such intimate union as to appear as one body. That this is so is indicated by several lines of evidence. In the first place, in cells of younger larvae or less advanced cells of old larvae, we may see various stages of this union. Just when the elements begin to unite and where the process starts, are questions of interest which have not, as yet, been worked out. However, there is some evidence which suggests that the process begins at the spindle fiber end. Thus, in the case of the "Mottled 5," while the greater part of the fourth chromosome is attached to the left end of the X, nevertheless, the normal fourth tends to join with the XL-IV component, at the region of the spindle fiber although little more than the spindle fiber end of the IV element is left.

That the chromosomes of Drosophila should exhibit a tendency to lie together in the spireme stages is not surprising. The recent work of HEITZ and BAUER (1933) shows this to be the case in the nuclei of the Malpighian tubules in *Bibio hortulanus*, and it has long been known that in Diptera, in general, homologues tend to lie together in all types of metaphase plates. This fact, of itself, is an indication of an attraction between homologues, or else that some highly developed cellular mechanism is responsible. If the attraction is chemical in nature, a moment's consideration of the facts will lead us to the conclusion that it is particulate and not a general mass reaction, for not only do like chromosomes lie together, but like parts are usually apposed. The astonishing feature is, as the present study has shown, that the attraction is so strong that it brings about an apparent fusion, and so specific that like bands or areas join up even though the form of the entire chromosome must be distorted in order to bring this about, as in the inversion figures. This brings us to a second line of evidence for union, and that is the complex figure we obtain when one element of a pair carries an inverted section. Inversion figures and mutual translocation constitute, to my mind, the most convincing evidence of the intimate nature of the chromosome union in these gland cells.

From a theoretical cytological standpoint, this intimate somatic synapsis is one of the most interesting features of the present study. Heretofore, synapsis has been thought of as a unique feature of germ cells approaching maturation. The present study has shown that in salivary gland cells we have a process which is externally similar. This raises a host of questions of particular interest to cytologists and would seem to offer a new avenue

of approach to the old problem of the cause for the behavior of the chromosomes during meiosis. What is the nature of this attraction between homologues in the salivary gland? What happens to the chromosomes during pupation and in the glands of the adult? What is the purpose of the union, and when and where does it begin? Does other somatic tissue show this besides the gland cells? How extensive is the process in other insects? All these questions await investigation, and while it would be hazardous to forecast the answer it seems safe to suggest that we will have new light on the processes which lead up to the maturation divisions in germ cells.

It should be pointed out that in *D. melanogaster*, after the union of homologues, we have six elements in the cell and not the haploid number as described by HEITZ and BAUER for *Bibio hortulanus*. In principle, however, my observations agree with these authors. In *D. melanogaster*, the two arms of the large V-elements show no obvious connection, each being embedded separately in the nuclear meshwork. In reality, of course, there is a somatic synapsis of homologous elements, and the reason the haploid number does not appear, is due to this structural peculiarity of the V-shaped chromosomes.

If it should be found that in somatic cells, in general, in Drosophila, homologues are as closely associated at the interphase as in the salivary gland cells, we can easily understand the mechanics of mottling which often results when one chromosome of a pair carries a "weak spot," as a result of a translocation or other causes. (See recent paper by PATTERSON 1933 in which such cases are described and pertinent literature is cited.) For, when homologues begin to untangle in the prophase a "weak" chromosome might easily tear in two. Likewise, it is obvious that if there were crossing over between homologues during somatic synapsis, induced by irradiation (PATTERSON, 1929), or occurring sporadically in normal tissue, we would have the physical basis for true somatic segregation. In this connection it may be noted that HEITZ and BAUER report the intimate association of chromosomes in Bibio in cells of the intestine, salivary glands and brain cells, as well as in the Malpighian bodies. However, whether this is simply a case of close apposition or not cannot be shown until inversions are studied.

Another point of interest, cytologically, is the fact that the spindle fiber ends of all the chromosomes (or arms for V-shaped elements) are apparently imbedded in a common mesh work. This seems to hint that there may be a very definite structural organization in the nucleus in the metabolic ("resting") phase. One wonders what relation this meshwork or coagulum bears to the spindle fiber mechanism. When the chromosomes (or arms) are torn free from the coagulum we often see at the tip of the spindle fiber end a small ring-like structure, two in paired chromosomes, and one

such alveolar body for single elements. These probably represent the point at which the chromosomes are attached to the mitotic spindle. Such structures have been widely reported in the past decade. If the interpretation given is correct, it would follow that *D. melanogaster* arose from a species of flies which carried 12 points of spindle fiber attachment, the two arms of the second or the third chromosomes, respectively, representing originally the rod-shaped chromosomes. The work of METZ (1916) indicates that 12 is the highest number of elements found in any species of this genus. Whether or not both arms of the second (or third) chromosome now possess separate attachment points I am not prepared to say, but this appears likely. The whole question of chromosome structure will be taken up later, at which time a full review of the current literature will be given.

LITERATURE CITED

BOLEN, H. R., 1931 A mutual translocation involving the fourth and X chromosomes of Drosophila. Am. Nat. **65**: 417–422.

DOBZHANSKY, Th., 1932 Cytological map of the X chromosome of *Drosophila melanogaster*. Biol. Zbl. **52**: 493–509.

HEITZ, E. and BAUER, H., 1933 Beweise für die chromosomennatur der Kernschleifen in die Knäuelkernen von *Bibio Hortulanus* L. Z. f. Zellforsh. u. mikr. Anat. **17**: 67–82.

METZ, C. W., 1916 Chromosome studies on the Diptera. J. Exp. Zool. **21**: 213–280.

MULLER, H. J. and PAINTER, T. S., 1932 The differentiation of the sex chromosomes of Drosophila. into genetically active and inert regions. Z.I.A.V. **62**: 316–365.

PAINTER, T. S., 1931 A cytological map of the X chromosome of *Drosophila melanogaster*. Science **73**: 647–648.

PATTERSON, J. T., 1929 Somatic segregation produced by X-rays in *Drosophila melanogaster*. Proc. Nat. Acad. Sci. Washington **16**: 109–111.

1933 The mechanism of mosaic formation in Drosophila. Genetics **18**: 32–52.

STERN, C., 1931 Zytologisch-genetische Untersuchungen als Beweise für die Morgansche Theorie des Faktorenaustausche. Biol. Zbl. **51**: 547–587.

CHROMOSOME CONSTITUTION AND BEHAVIOR IN EVERSPORTING AND MOTTLING IN DROSOPHILA MELANOGASTER*

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INTRODUCTION

Eversporting of somatic characters is found in nature in many plants and animals, in the form of variegated leaf, flower, or fruit color in plants, piebald coat color in animals, et cetera. Since every cell in an adult plant or animal is derived from a single germ cell by a process of cell division in which equal distribution of chromatin is supposed to take place, it is difficult to see how two adjacent cells in the petal of a flower, for example, can assume different colors. Two possibilities exist:—either (1) the genetic constitution is stable and differences are due to environmental variations; or (2) genetic constitution is unstable.

Unstable genetic constitution has been suggested as responsible for several cases of eversporting. Variegated pericarp in *Zea mays*, (EMERSON 1917), variegated flower color in *Delphinium ajacis*, (DEMEREC 1931), variable wing shape and eye color in *Drosophila virilis* (DEMEREC 1926, 1927), have all been attributed to unstable genes, genes which mutate with a definite frequency.

Eversporting eye color in *Drosophila melanogaster* is a common result of X-ray treatment. One case (PATTERSON 1932a) has been attributed to unstable genetic constitution, not an unstable gene in this case, however, but an unstable translocation of several genes to an abnormal position. Many other cases remain unexplained. Most of them involve chromosome translocations near one of two loci,—either the white region of the X chromosome, or the plexus to speck region of the second chromosome (MULLER 1930, VAN ATTA 1932). Factors of the latter type are dominant to normal red and produce a recessive lethal effect. Factors of the other type are recessive and require the presence of heterozygous white for somatic expression in females. These factors are usually lethal to males and when males do survive they are usually sterile. PATTERSON's case mentioned above belongs in this class of sex-linked mottled-eye colors. A third

* The cost of the accompanying color plates is paid by the GALTON AND MENDEL MEMORIAL FUND.

locus, ruby in the X chromosome, is involved in another type of mottled-eye color (GOWEN, unpublished).

This paper presents a study of three X-ray induced mottled-eye stocks. All are linked with the white region of the X chromosome and also with certain loci in the autosomes. Indications for and against different types of unstable genetic constitution are discussed. A genetic variable, presence or absence of an extra Y chromosome, is shown to have a profound effect on mottling. An environmental variable, temperature, is also shown to influence mottling.

Preliminary accounts of these mottled stocks and of the X-ray experiments from which they were derived have been published previously (GOWEN and GAY 1933a, 1933b, 1933c).

MOTTLED-1

A wild-type male, treated with 4460 electrostatic units of copper X-ray, was mated to a female whose two sex chromosomes contained *ClB*, and white (*w*) miniature (*m*) Beadex (*Bx*), respectively. One of their Bar daughters was mated to a *w m Bx* male. The offspring of this pair were mottled males, Bar Beadex females, and mottled Beadex females.

The *ClB* X chromosome has a dominant factor suppressing X chromosome crossing over, a recessive lethal, Bar, which serves as a marker for females carrying this chromosome, and several recessive factors.

The dark parts of the eyes of mottled-1 males are red or purplish and are smooth. The light parts are white or yellowish and are rough, the cells being set at different levels with deep spaces between cells. The appearance of the light areas in males suggests that many cells fail to function during the progress of embryological development. Eyes vary in amount of mottling from nearly wild-type to half white. The testis coloring shows a mottling of normal yellow pigment interspersed with white. The eyes of mottled-1 females are mottled with red or purplish and white or yellowish, the darker color generally predominating. The eye surface is smooth throughout. Except for eye color the female is wild-type in appearance. Mottled-1 male and female flies are shown in plate 1.

The presence of white (or one of its mutant allelomorphs) in the untreated X chromosome is necessary for the production of mottling in females. A female whose untreated X chromosome contains the normal allelomorph of white has red eyes and looks normal wild-type in every respect.

White-eyed flies are more viable than mottled-1, although white itself is less viable than wild-type. In cultures in which the two classes white and mottled are expected to be equal in number, the divergence from equality is between 25 and 50 percent for males, and 15 and 25 percent for females.

PLATE 1.—Mottled-1 male, and spermatogonial metaphase chromosomes. Mottled-1 female, and oögonial metaphase chromosomes. We are indebted to Dr. RALPH G. SCHOTT for the drawings.

This divergence indicates a high embryonic death rate due to the genetic complex causing mottled-1. The female death rate is less than the male, due we believe to the fact that females have a normal X chromosome which, as in recessive lethals, in some measure protects the organism, whereas the male does not have this protection.

The change from red to white never takes place early enough in development to produce flies whose eyes are all white, that is, mottled-1 does not overlap white. This is shown by a mating in which the normal X chromosomes are marked by yellow, white, echinus. Such overlapping would result in white, not-yellow, not-echinus flies, and none of these appear.

Linkage tests of mottled-1 show that it is completely linked with the first, second, and third chromosome factors white, Curly, and Stubble but not with the fourth chromosome factor Spread. That is, mottled-1 Curly Stubble females have offspring of only two recombination classes, mottled not-Curly not-Stubble, and white Curly Stubble; mottled-1 Spread females have offspring in all four possible classes, mottled, mottled Spread, white, and white Spread.

Tests involving other factors in the first, second, and third chromosomes show incomplete linkage with mottled-1. The sex chromosome change resulting in mottled-1 is found to the left of crossveinless. The recombination rates for the different regions of the X chromosome are:

	recombination	standard
yellow-echinus	0	4.4
echinus-crossveinless	2.8 ± 0.4	8.2
crossveinless-vermilion	15.7 ± 2.4	19.3
miniature-Beadex	18.7 ± 1.8	23.5

No crossing over occurs between yellow and echinus. The rate of interchange between echinus and crossveinless is too low. The rates in the other regions are not significantly divergent from the accepted standard ratios.

Mottled-1 has an apparent locus in the right limb of the second chromosome. Crossing over in the left limb is unaffected, but in the right limb it is so much reduced that the locus of mottled-1 cannot be determined. The following recombination rates are found:

	recombination	standard
dumpy to black	36.0 ± 1.2	35.5
black to curved	10.9 ± 0.8	27.0
curved to speck	4.0 ± 1.2	31.5
curved to mottled-1	0.1 ± 0.1	

As stated above the mottled-1 complex is completely linked with Stubble. The following recombination rates with other third chromosome factors are found:

	recombination	standard
Dichaete to mottled-1	15.3±0.7	29.1
mottled-1 to Hairless	9.3±2.3	
roughoid to hairy	27.6±1.3	26.5
hairy to mottled-1	25.8±1.3	44.2
mottled-1 to sooty	5.3±0.7	

Evidently mottled-1 is very close to the locus of Stubble at 58.2, and decreases crossing over slightly in its own vicinity. Other data demonstrate that crossing over between sooty, rough, and claret is not decreased in mottled-1 females.

Mottled-1 offers real technical difficulties in carrying the genetic analysis as far as we would like because of the fact that of the 200 or so males mated, not one was fertile. It has in a measure compensated for this difficulty, however, since triploids have been produced from two separate origins in this stock. Mottled-1 triploids whose two normal X chromosomes carry white, have eyes which are not mottled but are uniformly purplish colored. Triploids have been bred with the following results. Their sons are all white. Their diploid daughters are about 90 percent white-eyed, 10 percent red-eyed. Triploid daughters all resemble the mother. Intersexes are, with few exceptions, mottled. Mottled intersexes fall into two sharply differentiated classes, light mottled and dark mottled.

In diploid mottled-1 females, complete linkage was found between white, Curly, and Stubble. In triploids, white, Curly, and Stubble separate at random with respect to each other. It seems probable, however, that the first, second, and third chromosomes of the mottled complex remain completely linked in triploids. The data suggest that at maturation the two sets of normal chromosomes pair and segregate, whereas the chromosome group responsible for mottling does not pair but goes to one pole or the other as an independent unit. It would be of interest to know whether or not crossing over takes place between the normal and abnormal chromosomes of triploids. No information has been obtained on this point. If abnormal regions pair with normal regions, however, the progeny should have included mottled diploid females and mottled males. If all diploid eggs contain the mottled complex all the progeny should be either white diploid or mottled triploid. The latter is true except for two classes, white intersexes and red-eyed females.

A red-eyed female mated to white males, has white, mottled-1, and red-eyed progeny in a ratio of about 2:1:1. The constitution of red-eyed sports remained unknown until after the analysis of red eyes occurring in mottled-2 stock. That phenomenon could be definitely attributed to the presence of an extra Y chromosome. But when that problem had been solved our mottled-1 triploid stock had been lost. Reasoning by analogy, it seems very

probable that the red-eyed daughters of mottled-1 triploids are of the same constitution as ordinary mottled-1 diploid females with the addition of a Y chromosome. Further evidence in support of this reasoning will be shown later. The theory which best fits the facts is that a red-eyed female receives a Y and a set of autosomes from her father, and a normal X and the mottled X, 2nd, and 3rd triplet from her mother as a result of non-disjunction.

The flies classified as white intersexes might easily belong in the class of light mottled intersexes. The distinction between light mottled and dark mottled intersexes is extremely sharp with no overlapping, but light mottled tends to overlap white, so that an error in classification would be quite possible. The white intersexes could, then, represent non-disjunction or very light mottled flies classified as whites.

The difference between light mottled and dark mottled intersexes is so sharp that there must be a constitutional difference. Reasoning here again by analogy with mottled-2 it seems very probable that the light intersexes have no Y chromosome and that the dark intersexes have a Y chromosome.

To test the theory that an extra Y chromosome would inhibit loss of color from mottled-1 eyes, mottled-1 females were mated to white-eyed XYY males. Mottled-1 females mated to white-eyed XY males produce mottled and white offspring. Mottled-1 females mated to white-eyed XYY males produce red, mottled, and white offspring. Red-eyed females produced in this way are XXY, as is demonstrated by the appearance of secondary non-disjunctional exceptions among their offspring. This effect of the Y chromosome, common to all three of our mottled types, will be discussed later.

In cytological study of mottled-1, the most striking evidence of reorganization is the tangled mass in which the chromosomes are usually arranged. Only rarely are they separate and distinct enough for determination of their shape and number. When they can be studied, the individual chromosomes look normal except that in every case one of the large chromosomes is abnormally long and S-shaped. No other abnormalities have been detected, although genetic evidence shows that the first, second, and third chromosomes are all involved. Plate 1 shows spermatogonial and oögonial metaphase plates in which the chromosomes are unusually well separated. Multipolar cells are also found. These cells are large and may have as many as 24 or 32 chromosomes, the whole forming a kind of syncytium.

MOTTLED-2

A wild-type male given 13380 e.s.u. chromium radiation was mated to a *ClB* female. One of his Bar daughters, mated to *w m Bx*, had no sons: her

daughters were half Bar Beadex and half mottled Beadex. Later cultures produced mottled males in small numbers. Mottled-2 females have normal viability and fertility. Mottled-2 males have very low viability, but when they do survive they are fertile. Homozygous females are very rare and have not bred. Mottled-2 females are more viable than mottled-1 females. Mottled-2 males are much less viable than mottled-1 males but they are fertile whereas mottled-1 males are sterile. Typical mottled-2 flies have about seven-eighths of their eye area pale, thus being much lighter-eyed than mottled-1.

In mottled-2 as in mottled-1 the entire female eye has a normal smooth surface. The eye of a mottled-2 male is smooth in the dark areas but the pale areas are rough. Females homozygous for the X-rayed sex chromosome have eyes like those of mottled-2 males.

In addition to eye color and surface changes, mottled-2 produces other effects. Heterozygous females are often Notch. Males have mottled testis color. Males and homozygous females may have many bristles and hairs missing; several bristles may grow from one spot; wings may be spread or lifted; wings may be cut along the inner margin, or at the tip as in Notch females; wing veins may be thickened, branched and distorted. Mottled-2 male and female flies are shown in plate 2. The wings of the male are extremely modified. Typical male wings are more nearly normal.

Breeding tests show that mottled-2 is associated with the X and fourth chromosomes. Tests with Curly, Dichaete, and Stubble reveal no linkage with the second and third chromosomes.

The recombination rates for the different regions of the X chromosome are:

	recombination	standard
yellow-echinus	0	5.5
echinus-crossveinless	$.7 \pm 0.2$	8.2
crossveinless-miniature	16.2 ± 1.0	22.4
miniature-Beadex	19.3 ± 1.0	23.5

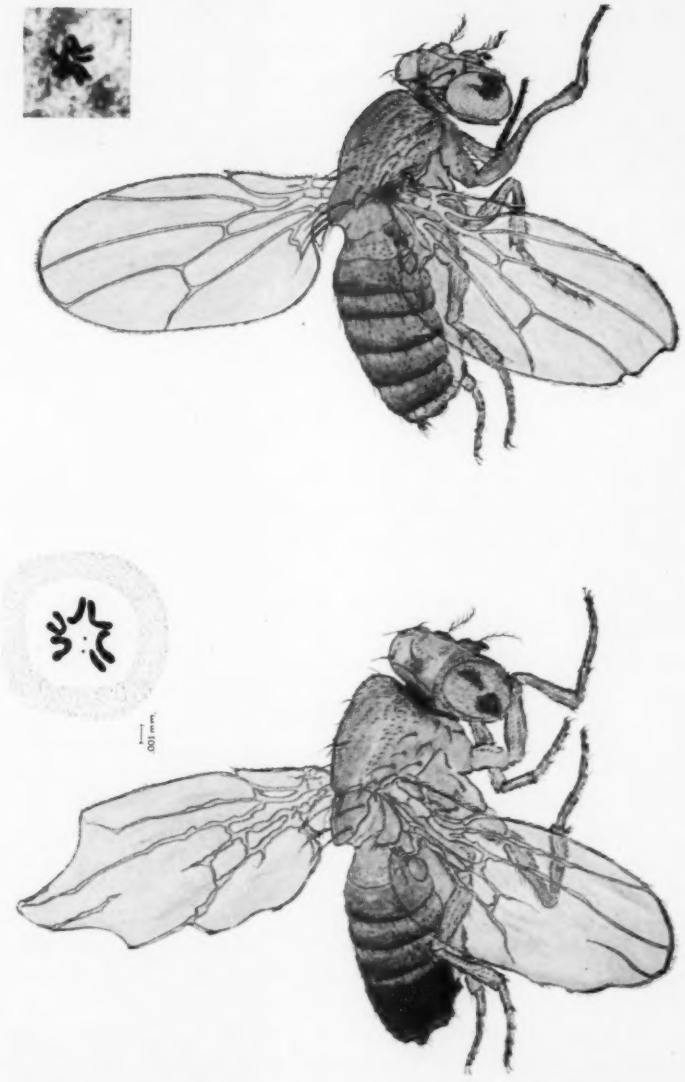
These data indicate that crossing over is completely suppressed left of echinus, much lowered between echinus and crossveinless, slightly lowered between crossveinless and miniature, and practically unchanged between miniature and Beadex.

Mottled-2 is completely linked with the fourth chromosome factors eyeless-2 and Spread.

When mottled-2 females are mated to white males, the progeny are white females, mottled females, and white males in about equal numbers, and a few mottled males. Non-disjunction of the fourth or X chromosomes is extremely rare, and hyperploid progeny have never been observed.

When mottled-2 males are bred they show several peculiarities not found

PLATE 2.—Mottled-2 male, and larval ganglion metaphase chromosomes. Mottled-2 female and oögonial metaphase chromosomes.



in the breeding behavior of females. A mating of mottled-2 males with white miniature females would be expected to produce white miniature males and mottled females. In addition to these types this mating produces (1) many *w m* Minute males, (2) a few females with wild-type red eye color, and (3) a few hyperploid males which are miniature and have mottled eyes. These exceptions can be explained by (1) non-disjunction of the fourth chromosomes (2) non-disjunction of the X and Y chromosomes, and (3) separation of the two segments of the broken X chromosome.

(1) Non-disjunction of fourth chromosomes. Minute males were mated to eyeless-2 females. Several offspring were Minute and eyeless-2, showing that they were haplo-IV. To test for triplo-IV female counterparts of these haplo-IV males, progeny from mottled-2 Spread females which had received the broken X from their father were examined. Some of these females had three fourth chromosomes, one Spread, one normal, and one attached to the X segment. Random segregation of the three fourth chromosomes resulted in apparent loss of linkage between mottled-2 and Spread. In all other flies tested, mottled-2 and Spread were completely linked.

(2) Non-disjunction of X and Y. XO male counterparts of the XXY females probably occur but have not been identified, because the high incidence of haplo-IV males makes the bobbed test for XO males impractical since haplo-IV and bobbed males are very similar. The breeding behavior of XXY females, and the fact that their eyes are red instead of mottled, will be discussed later.

(3) Separation of the two segments of the broken X. Hyperploid males have the smaller segment of the broken X chromosome in addition to a complete set of normal chromosomes. This condition is almost always lethal to males. A hyperploid fly, male or female, may have wings spread and lifted, wing veins irregular, bristles erect and easily broken, and eyes slightly roughened. Any or all of these characteristics may overlap normal, so that hyperploids are perfectly classifiable only if their normal X's are marked by recessive mutant genes, such as crossveinless or miniature whose normal allelomorphs are not included in the small broken X segment; for example hyperploid flies whose X chromosomes carry yellow white echinus crossveinless have mottled eyes and look crossveinless but do not look yellow or echinus. Hyperploid males and females are apparently normal in sexual characters, which indicates that the small segment of the broken X contains no sex-determining factors. Hypoploid flies, which might be expected as counterparts of hyperploids, have not been observed.

When mottled-2 males are mated to females with attached yellow white miniature (*y w m*) chromosomes their progeny differ markedly from the expected types, that is, mottled males, yellow white miniature females, and mottled superfemales. One such mating resulted in the following progeny:

regular	67 *y w m* females
	62 mottled males
	1 mottled superfemale
exceptional	19 *y w m* haplo-IV females
	2 red-eyed males
	2 *y w* superfemales
	30 dark mottled miniature hyperploid females
	8 light mottled miniature hyperploid females

The exceptions correspond exactly with the exceptions noted above.

(1) Non-disjunction of fourth chromosomes. Many of the yellow white miniature daughters are haplo-IV due to non-disjunction of the fourth chromosomes in the father. Triplo-IV males probably occur but have not been identified.

(2) Non-disjunction of X and Y. Most of the sons have mottled eyes, but males with red eyes are fairly frequently found. These are of the constitution XYY, the extra Y being due to non-disjunction of the X and Y in the father. Red-eyed males have much higher viability than mottled-2 males. A non-disjunctional female type, light mottled hyperploid, will be discussed under (3).

(3) Separation of the two segments of the broken X makes possible two exceptional types of females. One has the larger segment, the other has the smaller segment, of the broken X in addition to a complete set of normal chromosomes. The latter is a mottled miniature hyperploid; the former, yellow white, looks superfemale, and is apparently sterile. The non-disjunctional female type mentioned under (2) differs from her regular hyperploid sisters in eye color. A regular hyperploid receives a pair of attached X's from her mother, and a Y and the smaller X segment from her father. The eyes of such hyperploids are dark mottled. Non-disjunctional hyperploids have light mottled eyes. Their chromosomes are the same as those of their dark mottled sisters except that they receive no Y chromosomes from their fathers. This is demonstrated by mating the light mottled hyperploids to bobbed males. Their sons, receiving a paternal bobbed X but no maternal Y, then look bobbed. Bobbed is a factor which is visible only in the absence of a Y chromosome. Hyperploid females are fully viable and fertile, their sons being like the father except for rare hyperploids, and their daughters half normal and half hyperploid.

Hyperploid females without attached X's may be obtained by mating mottled-2 males to XXY females. Hyperploid females whose two normal X chromosomes carry yellow white echinus crossveinless look crossveinless and mottled, but neither yellow nor echinus. Hyperploid females whose two normal X's carry ruby miniature show both these factors. Therefore

the X-rayed X chromosome was broken between the loci of echinus and ruby,—between 5.5 and 7.5 units from the left end.

Linkage between mottled-2 and fourth chromosome factors must mean that one piece of the broken X is attached to the fourth chromosome. If the small segment were attached to the fourth, hyperploid females carrying Spread could have only two kinds of daughters, Spread and hyperploid. Actually such females have four kinds of daughters in about equal numbers, Spread, hyperploid, Spread hyperploid, and not-Spread not-hyperploid. Therefore the small X segment is not attached to the fourth chromosome.

Further evidence was obtained by mating mottled Spread males to yellow attached X females. If the fourth chromosome were attached to the small X segment, regular female offspring and yellow superfemales would be Spread; males, hyperploid females, and not-yellow superfemales would be not-Spread. If the fourth chromosome were attached to the large X segment, regular and hyperploid female offspring would be Spread; male and all superfemales would be not-Spread. That the fourth chromosome is attached to the large X segment is shown by the following progeny count:

females	151 yellow Spread
	91 hyperploid Spread
males	65 mottled
	6 red-eyed
superfemales	24 yellow
	14 not-yellow

It has been noted that crossing over is completely suppressed left of echinus and much lowered between echinus and crossveinless. The implications are that the small free segment of the broken X chromosome does not cross over with the normal X in diploids and that the reduction of crossing over at the ruby end of the larger segment of the broken X is due to the attachment of the fourth chromosome at that end. In a stock of triploids made by mating mottled-2 males to attached yellow triploids, a very low rate of crossing over was observed between yellow and mottled-2. That is in accordance with expectation, since crossover rates are generally higher in triploids than in diploids.

That non-disjunction was responsible for the repeated appearance of red-eyed females and males among the offspring of mottled males, was not readily apparent. No satisfactory explanation could be found from breeding the red-eyed individuals. Their progeny include red, mottled, and white-eyed flies. White and mottled flies from such matings, mated singly to white, breed true to type. Red-eyed flies, mated singly to white, repeat

the progeny ratios of the original red-eyed flies. Red-eyed flies are never Notch.

Cytological studies of the ovaries of mottled and red-eyed females suggested the probable explanation. Mottled-2 females have typical chromosome figures, but red-eyed females all have an extra chromosome in each figure. This extra chromosome looks like a Y chromosome. Plate 3 shows red-eyed male and female flies from mottled-2 stock and chromosome figures found in these flies.

Bobbed was introduced into mottled-2 stock, by crossing over, to test for the presence of a Y chromosome in mottled and red-eyed females. All mottled bobbed females look bobbed; no red-eyed bobbed females look bobbed.

Additional proof that red-eyed females have a Y chromosome is shown by the fact that red-eyed females have non-disjunctional progeny and mottled females do not. Of 223 offspring from red-eyed mothers 19 were non-disjunctional exceptions, whereas among 1937 offspring from mottled mothers, no exceptions occurred.

Cytological study of mottled-2 reveals chromosome figures which are normal in every respect in their appearance. This seems to indicate that the small free X segment closely resembles a normal fourth chromosome, and that the larger X segment with a fourth chromosome attached closely resembles a normal X chromosome. Presumably the small free segment has acquired a spindle fiber, since it does not become lost at cell division. If the larger X segment had two spindle fibers, one at each end, as it must have if it retained both its own and that of the attached fourth chromosome, it might be U-shaped with both ends pulled toward the center of the metaphase plate, but this abnormality has not been observed in mottled-2. There is no evidence that pairing of the larger X segment with the normal X is abnormal, as it would be if their spindle fibers were attached at opposite ends. Therefore we suppose that the spindle fiber attachment point has been broken off the X-rayed fourth chromosome and has become attached to the small free segment of the broken X chromosome.

The small free X segment and the normal fourth chromosome pair near the middle of the metaphase plate, apparently not attracted by their normal homologues which are at the distal ends of the X chromosomes. The figures suggest no explanation of the fact that the two segments of the broken X behave as an unbroken unit at oöcyte maturation but frequently segregate at spermatocyte maturation. Plate 2 shows male and female figures for mottled-2.

Hyperploid female oögonial figures differ from normal only in having

PLATE 3.—Red-eyed male from mottled-2 stock, and larval ganglion metaphase chromosomes. Red-eyed female from mottled-2 stock, and oögonial metaphase chromosomes.

.001 mm.

three chromosomes which resemble fourth chromosomes, of which two are normal fourths and the third is the small free X segment. One such figure is shown in figure 1.

The small free X segment is indistinguishable from a normal fourth chromosome. PATTERSON (1932a) and DOBZHANSKY (1932) find segments of similar genetic length to be three or four times the length of a fourth chromosome. DOBZHANSKY has demonstrated that the cytological length of the X chromosome between silver and kurz is rather long, so that the discrepancy may be due to the absence of an inert section from that region of our small X segment. No loss has been demonstrated by genetic tests. The normal allelomorphs of yellow, scute, silver, kurz, broad, prune and echinus are all present in our small segment, as shown by the fact that hyperploid females whose normal X chromosomes carry any of these mutant genes are always wild-type for these characters.

A point of interest in regard to scute was noticed in the course of this investigation. Hyperploid females whose normal X's carry scute occasionally lack one or two scutellar bristles, indicating that one wild-type allelomorph is not completely dominant to two scute genes.

MOTTLED-3

A wild-type male given 17840 e. s. u. chromium radiation was mated to a *ClB* female. One of his Bar daughters, mated to white males, had no sons except white ones due to non-disjunction. Three of her non-Bar daughters had mottled eyes. Mottled-3 females are of normal viability and fertility. Mottled-3 males have never been found.

Mottled-3 eye color is very variable, ranging in a single culture from as light as the lightest mottled-2 fly at one extreme to normal wild-type red at the other. Cultures vary in the proportion of mottled and red flies. Some cultures produce no mottled eyes, thus acting like ordinary sex-linked recessive lethal cultures.

Mottled-3 females are often Notch. Notching sometimes appears in flies with normal red eye color, but is more frequent in those with the most extreme mottling.

Mottled-3 is linked with the X chromosome and crosses over about 3 percent with Beadex, but not at all with miniature in 1000 flies. Examination of thousands of flies from matings of mottled-3 females, whose normal X chromosomes carried yellow white echinus crossveinless, to yellow white echinus crossveinless males, has never disclosed a crossover between mottled-3 and white, echinus, or crossveinless, but one mottled female with yellow body color appeared, representing, we suppose, a crossover between yellow and mottled-3. Two other possibilities exist however. A new mutation to yellow might have occurred; or the yellow locus may have become

deficient, allowing pseudo-dominance of yellow. Whatever the explanation of this one exceptional female, the crossing over rate between yellow and mottled-3 is extremely low.

Mottled-3 is completely linked with the second and third chromosome factors Curly and Dichaete. The following recombination rates for other factors in the second chromosome are found:

	recombination	standard
dumpy to black	31.2±1.3	35.5
black to mottled-3	9.2±0.8	27.1
mottled-3 to curved	5.1±0.6	
curved to speck	30.7±1.3	31.6

Evidently mottled-3 depresses second chromosome crossing over somewhat in its own vicinity, but not left of black nor right of curved. Its locus is about 65.

Third chromosome factor recombination rates are:

	recombination	standard
roughoid to hairy	30.3±1.3	26.5
hairy to mottled-3	10.4±0.8	44.2
mottled-3 to sooty	31.3±1.3	

Mottled-3 has no noticeable effect on crossing over in the third chromosome. Its locus is about 40.

Tests with Spread reveal no linkage between mottled-3 and the fourth chromosome.

Mottled-3 like mottled-1 does not produce hyperploids.

An attempt to increase the proportion of mottled eyes in mottled-3 cultures by selecting light mottled mothers showed such selection to be ineffective. A red-eyed female might have daughters just as light-eyed as daughters of a light-eyed mother; but most red-eyed females were found to have few or no mottled daughters. Primary non-disjunction of the X chromosomes occurs at a high rate in mottled-3 females. Red-eyed and mottled females whose normal X chromosomes carried white were mated singly to white Bar bobbed males to test for correlation between presence or absence of mottling and amount of non-disjunction.

In matings of that type, regular daughters are Bar, regular sons not-Bar. Non-disjunctional sons are Bar and look bobbed if they are XO due to primary non-disjunction, not-bobbed if they are XY due to secondary non-disjunction. Daughters are not-Bar if they are primary exceptions, Bar if secondary. Ordinarily it is impossible to distinguish between regular and secondary exceptional daughters except by further breeding. If mottled-3 were similar to mottled-1 and mottled-2 in the effect of an extra Y chromosome on eye color, regular females would be mottled and exceptional females would be red-eyed, whether primary or secondary excep-

tions. We should, in that case, expect regular females to be mottled Bar, primary exceptions to be red not-Bar, and secondary exceptions to be red Bar.

The progeny from twenty-two such matings show that mottled females are all XX, their exceptional sons being XO (look bobbed). Some red-eyed females are XX, having XO sons; other red-eyed females are XXY, having XY (not-bobbed) exceptional sons. The results of the twenty-two matings are summarized below:

15 mottled XX mothers	207 white sons
	45 white Bar bobbed sons
	204 white Bar daughters
	31 mottled Bar daughters
	188 red Bar daughters
	85 red not-Bar daughters
1 red-eyed XX mothers	21 white sons
	3 white Bar bobbed sons
	27 white Bar daughters
	15 mottled Bar daughters
	14 red Bar daughters
	19 red not-Bar daughters
6 red-eyed XXY mothers	111 white sons
	110 white Bar not-bobbed sons
	136 white Bar daughters
	105 red Bar daughters
	104 red not-Bar daughters

Obviously an extra Y chromosome is not the only factor inhibiting mottling of eye color in mottled-3 stock. The other factor was later found to be high temperature. If these twenty-two cultures had been raised at 18° Centrigrade, instead of in a variable room temperature, all the red Bar daughters of the sixteen XX mothers would have had mottled eyes since none of them can have had Y chromosomes; the XXY mothers would have had some mottled Bar XX daughters; but the not-Bar daughters in all cultures would still have had red eyes, because all must have received Y chromosomes from their fathers. Loss of color from mottled eyes is dependent upon temperature during larval life. This temperature effect is shown in less degree by mottled-1 and mottled-2 stocks. The effect of low temperature in increasing loss of color is dominated by the effect of the Y chromosome in preventing mottling.

Red-eyed XX females occasionally show Notch. Red-eyed XXY females are never Notch.

Cytological study of oögonial metaphase figures of mottled-3 females

reveals chromosomes which appear normal in size and number. But one of the large chromosomes is always oriented with one end toward the center of the metaphase plate, as if one or more translocations had made the spindle fiber attachment point terminal instead of median. Another large chromosome is often S-shaped. Figure 1 illustrates a typical mottled-3 figure.

MOTTLED-2 CROSSED WITH MOTTLED 1 AND MOTTLED-3

Since mottled-2 males are fertile it is possible to make crosses between mottled-2 and the other mottled stocks. When mottled-2 males are mated to mottled-2 females, a few homozygous mottled-2 daughters appear.

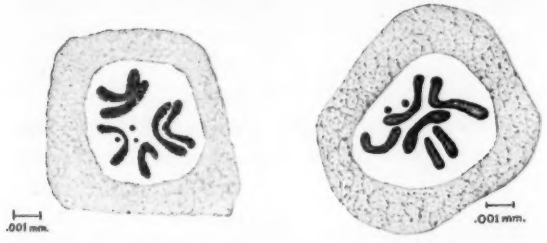


FIGURE 1.—A. Oögonial metaphase chromosomes of attached-X mottled-2 hyperploid female. B. Oögonial metaphase chromosomes of mottled-3 female.

These females, both of whose X chromosomes are broken, are rather inviable. Their eyes are like the eyes of mottled-2 males, both in color and in roughness of surface. When mottled-2 males are mated to mottled-1 or to mottled-3 females, a class of females corresponding to homozygous mottled females appears. These females, whose X chromosomes are both damaged by X-ray, have rough eyes. If the mother is mottled-1, the rough-eyed daughters are of normal viability. Their eyes may be red or mottled. If the mother is mottled-3 the rough-eyed daughters are rather inviable and thus far have had normal red eye color. It may be that at low temperature all these rough-eyed flies would have mottled eyes. The three kinds of rough-eyed females are of interest in suggesting a point of similarity between the three kinds of damaged X chromosomes.

DISCUSSION OF CAUSES OF MOTTLING

The amount of color in mottled eyes is dependent upon temperature, the lower the temperature at which the flies are raised the lighter their eye color (GOWEN and GAY 1933b). The range of variation at high temperature overlaps wild-type red occasionally in mottled-1, never in mottled-2, and very frequently in mottled-3. It has not been observed to overlap white,

even at very low temperatures, but mottled-2 and mottled-3 eyes are sometimes extremely pale. The effect of the Y chromosome is dominant to the effect of low temperature. When a culture is raised at 18° Centigrade, XX females and XY males have extremely pale eyes, but XXY females and XYY males have red eyes, or rarely red with a very few white ommatidia.

In all our mottled stocks the patches of dark and light ommatidia are more distinctly outlined in old flies than in young. The sharpness of the borders of the pigmented areas indicates that the production of pigment in a cell is determined by the constitution of that cell and that no diffusible substance seeps out from one cell to determine what the color of its neighbor might be. There is no evidence that the yellowish tinge of the light parts of mottled eyes is due to seepage of material from the dark areas because the line separating the two colors is distinct; and the yellowish color is evenly distributed throughout the light parts of the eye, not dependent upon the amount of red or purplish tissue in its vicinity.

A female whose sex chromosome composition is mottled/white has mottled eye color. If *W* is substituted for *w*, the female has red eyes, although in mottled-2 and mottled-3 she may show Notch. If a mutant allelomorph of white, such as champagne, eosin, or apricot is substituted for white, however, mottling occurs and the lighter parts of the eyes are the color of champagne, eosin, or apricot. Females homozygous for vermilion have mottled eyes in which the darker parts are vermilion in color. Males of the constitution mottled vermilion have mottled eyes in which the darker parts are vermilion. That is, in a mottled female the eye is composed of two colors; the darker color represents the genes actually present in her chromosomes and the lighter color represents the effect of pseudo-dominance of heterozygous white (or one of its mutant allelomorphs). In a mottled male the eye is likewise composed of two colors; the darker color represents the genes actually present in his chromosomes, but here the lighter color does not represent white pseudo-dominant because a male has only one X chromosome. The same condition is found in homozygous mottled-2 females; the darker parts of their eyes represent the genes present, but the lighter parts cannot represent pseudo-dominant white because neither X chromosome contains white.

The eye colors of mottled females heterozygous for white, champagne, eosin, and apricot suggest that mottling may be due to loss, inactivation, or mutation of the normal allelomorph of white in those ommatidia which show the lighter color. Whatever mechanism is responsible for mottling of eye color, it does not occur in germ cells, or if it does it is lethal; it is not lethal to somatic cells because the light areas in male and homozygous female eyes survive; it does not cause mottling for any other factor tested including yellow, scute, silver, kurz, broad, prune, echinus, ruby, cross-

veinless, vermilion, miniature, forked, and bobbed; it does often cause Notch in mottled-2 and mottled-3; it is partly or completely inhibited by the presence of an extra Y chromosome; and it is partly or completely inhibited by high temperature.

Red-eyed flies from mottled stocks are in many ways suggestive of sports found elsewhere, although the parallelism is not complete. Variegated pericarp color in corn is usually somatic in its effect, and striped kernels give rise to plants bearing white or variegated kernels. But often the sporting extends to the germ cells and a striped kernel will give rise to a plant with solid red kernels. These solid red kernels seldom yield further variegated stock. In a striped variety of snapdragon, seeds from either striped or red flowers will produce both striped and red flowers in the next generation, with definite frequencies (DE VRIES 1905).

A change in somatic tissue produces variegation, striping, or mottling. A similar change in germinal tissue produces a sport which may breed true, as does corn with very few exceptions; or which may be subject to further eversporting, as in snapdragon and these mottled eye stocks. There is some evidence that environmental conditions may be important in determining the distribution of color in variegated corn (EYSTER 1926), but DEMEREC (1932) found no effect of temperature on variable miniature in *Drosophila virilis*.

The experimental evidence presented in this paper shows that temperature and the Y chromosome can play very important rôles in controlling mottling and ever-sporting, and it seems possible that these factors may aid in analyzing other cases of eversporting thus far unexplained.

PATTERSON'S (1932a) explanation of one of his mottled stocks as due to an unstable translocation encounters several obstacles when applied to our stocks. Since mottled-1 and mottled-3 do not produce hyperploids, we have no way of locating the break in the X chromosome or even of demonstrating genetically that the X chromosome has been broken. The disturbed linkage relations between factors in the first, second, and third chromosomes make translocations seem very probable, however. In mottled-2 we can locate the X chromosome break definitely between echinus and ruby. It would be logical to suppose that mottling is due to the loss of the small X fragment from those cells which show lighter eye color, but in that case other factors whose normal allelomorphs are in the small free X fragment should mottle in a female whose unbroken X chromosome carries such mutants. For example, a female whose normal X carries *y w ec* might be expected to show mottling for yellow and echinus as well as white, especially echinus since white and echinus are both visible in cells of the eye. But yellow and echinus do not mottle, remaining completely recessive.

PATTERSON (1932 b) has demonstrated a viability factor between scute and prune, deficiency for which results in death of any cell in which it occurs. Since our small X fragment contains the normal allelomorphs of yellow, scute, silver, kurz, broad, prune, white, facet and echinus, presumably it contains all the genes which normally lie between yellow and echinus. If that were true the loss of the X fragment could be ruled out as a cause of mottling for the light colored cells would then be deficient for the viability factor. Two alternatives are possible—either our X fragment does not contain the viability factor or mottling is not due to loss of the fragment.

If mottling is not due to the loss of the whole X fragment it might be due to the loss of a part. We have shown evidence that the normal allelomorphs of yellow, scute, silver, kurz, broad, prune, echinus, and the viability factor are not lost. The incidence of Notch in many mottled-2 flies indicates loss of the facet locus. Therefore the explanation of mottling might be loss of the white-facet region without loss of the two ends of the fragment.

If the loss of the white-facet region of the X chromosome is the mechanism responsible for mottled eyes, the presence-absence hypothesis is confirmed in that the white ommatidia in mottled males are white because a gene in this locus is absent. Of course this would not indicate that white is always due to absence of red, but merely that absence of red does produce white.

Although we do not know what chromosome rearrangement is present in mottled-1 and mottled-3 stock, we can apply some of the foregoing reasoning. The white cells in the eyes of mottled-1 males are viable, so that deficiency for the locus of the viability factor in these cells is improbable. Since mottled-1 females never show Notch, and since they show mottling for no factors other than white, any loss of chromatin must be confined to the region very near white. Mottled-3 differs from mottled-1 and mottled-2 in that it is lethal to males. Therefore we need not exclude the possibility that any chromatin subject to loss might contain the viability factor. In mottled-3 Notch often appears in females, but mottling has not been observed for any other factor so that loss of chromatin at mitosis may include the loci of white and facet but no other tested loci.

No theory of the cause of mottled eye color in our stocks is really adequate. An alternative to loss of chromatin as an explanation might be an easily mutable gene in the white locus like the mutable genes found in *Drosophila virilis* by DEMEREC. The chief objection to such a theory is the difficulty of explaining the inhibitory effect of an extra Y chromosome.

The effect of an extra Y chromosome on mottling of eye color is no easier to understand than the cause of mottling itself, since the Y is in most respects so inert in comparison with the other chromosomes. The

fact that the Y has the same effect in all our mottled stocks suggests that some basic principle is involved.

If mottling is due to the loss of the region of the X-rayed X chromosome carrying the normal allelomorph of white then the Y chromosome might conceivably act in two ways:—(1) it might stabilize the process of mitosis by its mere presence, preventing the loss of chromatin, or (2) it might contain some gene-like material tending to be dominant to white. Evidence in favor of the former is furnished by the fact that red-eyed (XYY) males are much more viable than mottled (XY) males; which agrees rather with a stabilizing effect of the Y chromosome on chromosome balance, than with a genic influence on eye color. The state of chromosome rearrangement responsible for mottled eyes decreases the survival value of males unless the harmful effects are held in check by an extra Y chromosome. The fact that XYY males are more viable than XY males is significant in demonstrating the ecological importance of genetic constitution.

Since an extra Y chromosome makes mottled-1 and mottled-2 males much more viable, it might have been expected to make mottled-1 males fertile, or to increase the viability of mottled-3 male zygotes to the point of producing a few viable males. But no mottled or red-eyed males have ever appeared in mottled-3 cultures, and none of the red-eyed males from mottled-1 cultures have proved fertile.

Since an extra Y chromosome prevents mottling of eye color it might have been expected to prevent the irregularities in maturation so often shown by mottled-2 males. But red-eyed males from mottled-2 stock produce haplo-IV and hyperploid offspring, demonstrating that the extra Y chromosome does not stabilize meiosis, whatever its effect on mitosis may be.

There is some basic difference between maturation of spermatocytes and maturation of oöcytes in Drosophila. This is shown in normal individuals by the fact that crossing over occurs in females but not in males. Further evidence of difference is found in *c III G* stock (GOWEN 1928, 1933). The factor *c III G* is a third chromosome recessive which influences maturation in females, resulting in almost complete suppression of crossing over in all chromosomes, in high rates of non-disjunction of the X and fourth chromosomes, and in increased production of diploid ova. In spite of these irregularities in homozygous females, homozygous males are absolutely regular in every respect. An equally striking difference between male and female maturation is displayed by mottled-2 stock. As noted before, offspring of mottled-2 males exhibit three kinds of irregularity:—non-disjunction of X and Y chromosomes, non-disjunction of fourth chromosomes, and separation of the two parts of the broken X chromosome. Mottled-2 females are regular in all these respects. The X and fourth chromosome

rearrangement in mottled-2 stock is so abnormal that the irregularity of maturation in male germ cells is not surprising. It is, rather, the regularity of maturation in mottled-2 female germ cells which constitutes a problem, the solution of which may furnish a clue to the nature of the maturation process.

SUMMARY

Three X-ray induced mottled eye stocks are described. Males have rough mottled eyes. Females heterozygous for mottled and for white have smooth mottled eyes.

Mottled-1 is linked with the first, second, and third chromosomes. Males are somewhat less viable than normal and are sterile. Notch does not appear. No hyperploids or hypoploids are produced. Eye color never overlaps white but occasionally overlaps wild-type red, the average eye being about half red and half white.

Mottled-2 is linked with the first and fourth chromosomes. The X chromosome is broken between the loci of echinus and ruby; the smaller segment is free, and the larger segment is attached to the fourth chromosome at its left or ruby end. Males are of very low viability but are fertile. Males and females often show Notch. Males produce non-disjunctional and hyperploid offspring; females do not. Hyperploid males rarely survive. Hyperploid females are of approximately normal viability and fertility. No hypoploids are produced. Eye color never overlaps white or wild-type red, the average eye being about one-eighth red and seven-eighths white.

Mottled-3 is linked with the first, second, and third chromosomes. It is completely lethal to males. Females often show Notch. No hyperploids or hypoploids are produced. Eye color never overlaps white but very often overlaps wild-type red. Amount of mottling varies from about 90 percent white to all red.

Mating mottled-2 males to mottled females of each type produces females which are $\frac{\text{mottled-1}}{\text{mottled-2}}$, $\frac{\text{mottled-2}}{\text{mottled-2}}$ or $\frac{\text{mottled-3}}{\text{mottled-2}}$. Their eyes are rough like eyes of mottled males.

The amount of color in mottled eyes is dependent upon temperature.

There is no evidence of diffusion of color from red to white ommatidia.

Mutant allelomorphs of white may be substituted for white in heterozygous mottled females resulting in mottled eyes whose lighter areas assume the mutant color. Vermilion may be introduced into mottled stock, resulting in mottled eyes whose darker areas are vermilion.

The possible causes of mottling are discussed, but no satisfactory conclusion is reached. If mottling is due to the loss of the normal allelomorph for red eyes from the X-rayed X chromosome at mitosis in cells of mottled

eyes the presence-absence hypothesis is confirmed to the extent that the white ommatidia of mottled males are white because the gene for red is absent.

Whatever is responsible for mottling, its effect is inhibited by the presence of an extra Y chromosome. XYY males and XXY females have wild-type red eyes, not mottled, whether from mottled-1, mottled-2, or mottled-3.

Mottled-2 offers new evidence on the differences between spermatogonial and oögonial maturation processes in Drosophila, since meiosis is regular in females, irregular in males.

LITERATURE CITED

DEMEREC, M., 1926 Miniature-α—a second frequently mutating character in *Drosophila virilis*. Proc. Nat. Acad. Sci. Washington **12**: 687–690.

1927 Magenta-α—a third frequently mutating character in *Drosophila virilis*. Proc. Nat. Acad. Sci. Washington **13**: 249–253.

1931 Behavior of two mutable genes of *Delphinium ajacis*. J. Genet. **24**: 179–193.

1932 Effect of temperature on the rate of change of the unstable miniature-3-γ gene of *Drosophila virilis*. Proc. Nat. Acad. Sci. Washington **18**: 430–434.

DE VRIES, HUGO, 1905 Species and varieties. pp. 315–322. Chicago: The Open Court Publishing Co., 847 pp.

DOBZHANSKY, TH., 1932 Cytological map of the X chromosome of *Drosophila melanogaster*. Biol. Zbl. **52**: 493–509.

EMERSON, R. A., 1917 Genetical studies of variegated pericarp in maize. Genetics **2**: 1–35.

EYSTER, W. H., 1926 The effect of environment on variegation patterns in maize pericarp. Genetics **11**: 372–386.

GOWEN, J. W., 1928 On the mechanism of chromosome behavior in male and female Drosophila Proc. Nat. Acad. Sci. Washington **14**: 475–477.

1933 Meiosis as a genetic character in *Drosophila melanogaster*. J. Exp. Zoöl. **65**: 83–106.

GOWEN, J. W. and GAY, E. H., 1933 Eversporting as a function of the Y chromosome in *Drosophila melanogaster*. Proc. Nat. Acad. Sci. Washington **19**: 122–126.

1933 Effect of temperature on eversporting eye color in *Drosophila melanogaster*. Science **77**: 312.

1933 Gene number, kind and size in Drosophila. Genetics **18**: 1–31.

MULLER, H. J., 1930 Types of visible variations induced by X-rays in Drosophila. J. Genet. **22**: 299–334.

PATTERSON, J. T., 1932 A new type of mottled-eyed Drosophila due to an unstable translocation. Genetics **17**: 38–59.

1932 A gene for viability in the X chromosome of Drosophila. Z.I.A.V. **60**: 125–136.

VAN ATTA, E. A., 1932 Genetic and cytological studies on X-radiation induced dominant eye colors of Drosophila. Genetics **17**: 637–659.

A COMPARISON OF THE FREQUENCIES OF VISIBLE MUTATIONS PRODUCED BY X-RAY TREATMENT IN DIFFERENT DEVELOPMENTAL STAGES OF DROSOPHILA

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INTRODUCTION

The problem of the production of mutations by artificial means has been partially solved. The scientific world was apprised of this fact when MULLER (1927) offered convincing evidence that irradiations are instrumental in producing heritable variations in Drosophila. Other investigators, experimenting with other forms of genetic material, have obtained similar results following treatment of the germ cells with irradiations. Thus STADLER, at the UNIVERSITY OF MISSOURI, induced gene mutations in barley and corn by means of X-rays and radium. Subsequently, P. W. and A. R. WHITING obtained positive results by the use of X-rays on Habrobracon. From the field of plant genetics BLAKESLEE and BUCHHOLZ offer a mass of interesting mutation results from X-rays and radium applied to the Jimson weed, Datura. The findings reported by the above, and many other workers who have resorted to the use of irradiations as a means of inducing changes in the gene, consist of an abundance of hereditary effects similar to every type of heritable variation obtained in untreated material. It can no longer be questioned that the effects which X-rays and radium produce upon genetic material are of the same nature as those produced by natural causes, whatever the natural causes may be.

In subsequent pages of this paper I hope to submit significant data upon the relative frequencies of the production of visible mutations in *Drosophila melanogaster* treated with X-rays at different stages of development, and to offer some evidence concerning the nature of the genic material of the chromosome.

MATERIALS AND METHODS

In the interest of simplicity it was deemed wise to use only those changes in the genes of the sex chromosomes of Drosophila as a criterion of the rate of mutation. Since most mutations are recessive to the normal, it is much easier to detect them if they occur in the sex chromosomes than if they are produced in the autosomes. The experiments were made critical by obtaining a recessive autosomal gene, in homozygous condition, in the treated flies. This permitted subsequent tests for contaminations.

To secure a stock suitable for the experiment, one male and one female, taken from a culture of brown-eyed flies were mated in a yeast culture. By this procedure a stock of flies was obtained having all the genes normal, except for the recessive mutant located in the second pair of chromosomes which is responsible for the brown eye-color. This latter gene, being homozygous in both males and females, was used as a marker against contamination of the cultures. Virgin females for testing the frequency of visible mutations produced by treating mature ova were obtained from this stock, treated with X-rays, and mated immediately to males, the X chromosome of which contained the mutant genes for scute, vermilion and forked. Any male offspring from this cross would receive its X chromosome from the treated mother and also the recessive gene for brown eyes. If a recessive, or a dominant mutation was produced in the sex chromosome, or a dominant in one of the autosomes, it would find expression in the male offspring receiving the mutant gene. When no mutations were produced, all the male offspring appeared normal.

In order to obtain information on the frequency with which mutations are induced by irradiating mature spermatozoa, young males were taken from the stock of brown (at the same time the virgins were obtained), treated with X-rays, and mated to yellow females having the X chromosomes attached together at the right hand ends and containing, in addition, a supernumerary Y chromosome. All male offspring from this cross resulted from the fertilization of eggs carrying the Y chromosome of the mothers by the X-bearing sperm cells of the treated fathers. These males also received one second chromosome from the treated male that contained the recessive gene for brown eye-color. Any abnormalities due to the mutation of genes in the treated X chromosome appeared only in the male offspring.

The method employed for securing immature germ cells for treatment was designed to enable the experimenter to procure masses of cells of a definite age,—theoretically in the same stages of development. Mass cultures of "brown" flies were made in vials. Yeast food was introduced into the vials on small paper spoons. Immediately after placing the flies in the vials a spoon of fresh food upon which a drop of dilute acetic acid had been spread to induce egg production, was inserted into each tube and allowed to remain for thirty to forty minutes. During this interval any fertilized eggs undergoing development in the oviducts of the females should have been deposited upon the food. At the end of this period those spoons were replaced by spoons of fresh food to which dilute acetic acid had been added. The females were permitted to deposit eggs upon this food for a period of one hour. The culture medium bearing the eggs was then removed from the spoons and placed in small culture dishes containing

yeast food where development of the larvae continued until the time for treatment. All developing individuals obtained by this method were within one hour of the same age. These developing cultures were irradiated at the various ages deemed desirable and the culture media transferred to pint milk bottles to await the development of imagoes. Males and females were separated before copulation occurred and the individuals were mated as in the case of treated adults.

Six experimental groups were examined for visible mutations: (1) females treated as freshly hatched adults, (2) females treated at the larval age of 71–72 hours, (3) females treated in the 35–36 hour larval stage, (4) males irradiated as adults, (5) males treated at 71–72 hours of age, and (6) males treated as 35–36 hour larvae. A series of controls consisting of untreated "brown" females mated to "scute-vermilion-forked" males, and "brown" males crossed to the yellow females, was reared as a check upon the mutation rate. Experiments upon each group were conducted simultaneously to avoid any unsuspected environmental influences and to distribute equally, among all the groups, any errors which might result from inexperience in observation.

All environmental conditions were controlled as accurately as possible. The age at which the flies were irradiated, or the stage of development of the germ cells at the time of treatment, was the only variable in the process. When variations appeared among male offspring of treated, or control individuals, the variant males were mated to a stock of females which gave all sons like the fathers with respect to the X chromosome.

EXPERIMENTAL RESULTS

Mutations

Frequency of Mutations in the X Chromosome

Experiments with which this paper treats were devised to insure constancy of all factors, except the stage of development of the germ cells at the time of irradiation. Since MULLER (1930) reported an absence of heritable variations when females were rayed while larvae three to four days old, it seems logical that the transmuting power of X-rays is largely if not entirely lost upon immature eggs. It was hoped that some cogent evidence upon the frequency of mutations in the sperm and egg might be obtained by observing large numbers of F_1 individuals from both males and females treated at the same time and with equal intensity. In other words, it offers the possibility of ascertaining the effect of sex upon mutability.

The sex-linked heritable variations and the calculated mutation rates expressed in percentage are tabulated in table 1. No more than a glance at the table is necessary to learn that no sex-linked heritable variations were found among the 12,633 F_1 male offspring of untreated control females.

Few more appeared among the 13,673 sons of the control males; only one sex-linked mutation was discovered. When the visible mutation rate is calculated for this group it appears as .007 ± .005 percent, a very high natural mutation rate as compared with one of .002 percent based upon examinations of 20,000,000 flies (MULLER 1929).

TABLE 1

Mutation frequencies in the sex chromosome of treated and control flies and a comparison of mutation frequencies in sex.

SEX	AGE AT TIME OF TREATMENT							
	ADULTS		71–72 HOUR LARVAE		35–36 HOUR LARVAE		CONTROLS	
	MALE	FEMALE	MALE	FEMALE	MALE	FEMALE	MALE	FEMALE
Number of F_1 flies	11,620	12,525	7,677	12,237	6,945	7,600	13,673	12,633
Number of sex-linked mutations	33	23	11	13	5	11	1	0
Percent of mutations	.282 ± .034	.183 ± .026	.143 ± .029	.106 ± .018	.070 ± .020	.144 ± .028	.007 ± .005	.000 ± .000
Mutation frequency less control frequency	.276 ± .034	.183 ± .026	.136 ± .029	.106 ± .018	.063 ± .020	.144 ± .028	—	—
Difference divided by probable error	8.1	7.0	4.7	5.0	3.0	5.1	—	—
Difference in percent of mutations in sexes	.093 ± .042		.030 ± .032		.081 ± .034			
Difference divided by probable error	2.21		0.94		2.39			

A perusal of the data from the treated groups is sufficiently convincing as to the effectiveness of X-rays in producing gene mutations even when administered in the light "dose" (1325 r units) employed. In the course of the experiment 11,620 F_1 males, offspring of treated adult males, were examined for mutations. Of the variant individuals detected, thirty-three contained heritable sex-linked mutations. Therefore, the rate of mutation of X chromosomal genes for this group appears as .282 ± .034 percent, deviating from the percentage found in the control males by .276 ± .034 percent. The mutations obtained by irradiating adult females were slightly, but not significantly, less numerous. The 12,525 male offspring of females treated as adults contained twenty-three individuals that bred true as sex-linked mutations. The twenty-three mutant individuals represent .183 ± .026 percent of the total F_1 males.

A total of 7,677 F_1 males, offspring of males treated while larvae of from 71–72 hours of age, was examined for mutations. A large number of variant males were observed but only eleven, or .143 ± .029 percent bred true as X chromosomal mutations. When the percentage of mutations detected in control males is deducted, the remainder is .136 ± .029 percent, a differ-

ence 4.7 times the probable error. Females, treated as larvae of the same age, produced 12,237 F_1 males, thirteen of which were mutants. This number constitutes $.106 \pm .018$ percent in favor of the treated group. The difference, when comparison is made with the percentage found in controls, is 5 times its probable error.

Males treated at the larval age of 35–36 hours produced 6,945 F_1 male offspring. Of this number, five, or $.07 \pm .02$ percent developed from mutant sperm cells. This mutation percentage differs from that of the control males by $.062 \pm .0203$ percent. In this case the difference is only 3 times the probable error, a difference large enough to be generally accepted as significant. Females treated as 35–36 hour embryos gave 7,600 F_1 males, eleven, or $.144 \pm .028$ percent were mutants. Comparing this percentage to that found in control females the difference becomes 5.1 times the probable error and highly significant.

A Comparison of the Mutations in Sex

If the mutation rate obtained by treating a given group of males is compared with the rate observed when females of the same age are irradiated, no significant difference is observed. The comparisons of the sexes may be found in table 1. When the mutation frequency found for treated adult males is compared with the frequency obtained by testing females treated simultaneously, the difference is $.093 \pm .042$ percent; the difference, 2.21 times the probable error, is obviously without meaning. When the mutation frequency observed in males treated as 71–72 hour larvae is compared with the frequency occurring in females treated at the same age, the rate appears $.030 \pm .032$ percent higher in the males. The difference is less than the probable error. Males from larvae treated at 35–36 hours of age produced $.063 \pm .0203$ percent more mutant cells than were produced by the control males. Females, treated as larvae at the age of 35–36 hours, contained $.144 \pm .028$ percent more mutant germ cells than untreated controls. In this case irradiation failed to increase the rate of mutations more in one sex than in the other, the difference being $.081 \pm .034$ percent, or 2.39 times the probable error.

Comparison of the Mutation Frequencies of Different Groups of the Same Sex

In order to determine whether or not the age of the germ cell at the time of treatment plays any part in the mutability of the gene, the mutation frequencies of the different developmental groups of the same sex are compared. The results of these comparisons are briefly summarized in table 2. Males treated as adults gave $.140 \pm .044$ percent more mutations than males treated at the larval age of 71–72 hours. The difference, 3.20 times

the probable error seems to signify that mature sperm cells mutate more readily than immature cells of old larvae. The percentage of visible sex-linked mutations produced by irradiating adult males is .213 ± .037 percent higher than the percentage found when males are treated as 35–36 hour larvae. This difference, 5.6 times the error, is evidence of the detectable

TABLE 2

A comparison of the mutation frequencies of the different treated groups.

AGE AT TIME OF TREATMENT	SEX	PERCENT MUTATIONS BY X-RAYS	ADULTS COMPARED WITH 71–72 AND 35–36 HOUR LARVAE	35–36 HOUR COMPARED WITH 71–72 HOUR LARVAE	DIFFERENCE DIVIDED BY PROBABLE ERROR
71–72 hour larvae	Male	.136 ± .029			
			.140 ± .044		3.20
Adults	Male	.276 ± .034		.072 ± .035	2.05
			.213 ± .037		5.75
35–36 hour larvae	Male	.063 ± .020			
71–72 hour larvae	Female	.106 ± .018			
			.077 ± .030		2.56
Adults	Female	.186 ± .026		.035 ± .031	1.13
			.042 ± .037		1.14
35–36 hour larvae	Female	.144 ± .028			

increase of mutability of the gene residing in a mature sperm cell. If the mutability of the germ plasm of the two groups of males rayed as larvae are compared, no difference in the mutation frequencies is found.

An examination of the mutation frequencies produced by treating females in different stages of development reveals no differences in the frequency of gene mutation, irrespective of the stage of development irradiated. Details of the various mutation frequencies may be obtained from the table.

"Partial" and "Radical" Mutations

The term "partial" mutation, as applied herein, refers to cases in which the mutation failed to show in all parts of the body of the organism that should have been affected by the mutant character; to individuals having mutant somatic cells but normal germinal tissues; or to flies which produced part mutant and part normal offspring. According to definition those flies apparently having the tissue mutant, and giving only mutant offspring if not sterile, are considered as "radical" mutations. The terms "partial" and "radical" are employed since they are descriptive of the amount of

genic material undergoing change at a given locus. In case a "radical" mutation occurs, all the genes at a given locus of the various chromatin threads of the chromosome undergo mutation, while a "partial" mutation involves only a part of the chromatin at any given locus.

"Partial" mutations were brought to attention by phenotypically mosaic individuals which bred as "radical," or complete mutations—giving offspring which showed the mutant character in all regions of the body in which the mutation was capable of phenotypic expression. For example, one male, obtained from mating a treated male to a female having attached X's and a Y chromosome, was selected for breeding because of the absence of the right postvertical bristle. All other bristles were present. All the male offspring produced by the mating of this male to a double yellow female showed scute-1. In this case the somatic cells covering the area of scute-1, except for the region of the right postvertical, were normal but all the germ cells contained the mutant gene. Bearing in mind that only the variations appearing phenotypically the same as those known to be dependent upon gene mutation are to be considered, it is possible to divide the anomalous individuals into groups of "radical" mutants, one-half mutant, one-fourth mutant, and "minute" mutations (individuals in which the character is visible in less than one-fourth the somatic tissues capable of expressing the mutant gene). The proportion of "partials" to "radicals" may then be obtained; the sterility of the types compared, and a comparison may be made of the numbers breeding true in each group. Table 3 is a composite of all the data obtained from the different groups.

TABLE 3

Classification of variants obtained from treated groups.

TIME OF TREATMENT	"RADICAL" MUTATIONS			"PARTIAL" MUTATIONS										
				1/2-MUTANT				1/4-MUTANT				"MINUTE" MUTATIONS		
	NO.	ST.	T.	NO.	ST.	T.	N.T.	NO.	ST.	T.	N.T.	NO.	ST.	T.	N.T.
Adult males	98	59	39	103	21	11	71	4	0	1	3	8	0	1	7
Adult females	77	55	22	92	20	12	60	3	1	0	2	6	3	0	3
71–72 hour males	27	15	12	29	9	0	20					2	1	0	1
71–72 hour females	34	26	8	42	7	2	33	2	0	2	0	5	0	0	5
35–36 hour males	18	11	7	22	8	2	12	2	1	0	1	1	0	0	1
35–36 hour females	27	17	10	25	4	1	20					4	1	1	2

No.—Number of cases; St.—Sterile; T.—Bred true for mutant character, and N.T.—Failed to breed true

DISCUSSION

Mention has been made of the production of visible mutations in the X chromosome and the percent of mutations per treated group has been

noted in the foregoing section. By way of introducing a discussion of the mutation frequencies we may refer to table 1 for a review of the data. The mutation rate is increased in all the treated groups. The slightest effect is noticed when males are treated as 35–36 hour larvae, but the rate of mutations observed among offspring of this irradiated group is sufficiently high to be significant, the rate differing from that of the control males by $.063 \pm .0203$ percent,—a difference 3 times the probable error. A much greater increase in the mutation rate is observed when adult males are treated. The percentage of mutations found among offspring of males irradiated as adults is $.276 \pm .034$ percent above that of the control males. This figure is 8.1 times the probable error. As may be ascertained from table 1, the mutation rates found for all other groups are significantly higher than the rate observed in the control groups.

It is interesting to note that HANSON (1928) reported a visible mutation rate, obtained by treating mature males with a dose of X-rays equivalent to that employed by the writer, ten times that herein reported for males treated as adults. The apparently conflicting results can be brought to agreement by considering, in this experiment, the gene mutations that bred as such, irregularities resulting from chromosomal abnormalities, the anomalous sterile flies, and the aberrant flies that bred as normals under the heading of visible mutations. When such classes are grouped as visible mutations the rate becomes practically the same as that found by HANSON.

It has been observed repeatedly that the rate of induction of lethal mutations in mature sperm is much higher than in spermatogonia treated in the adult male but no comparative results, obtained by irradiating different developmental stages of cells in the female, have been reported. The visible mutations of the present experiment throw light upon the relative susceptibility of genic material, in different stages of development, to the transmuting power of irradiations. In male cells the rate of induction is apparently much higher when the mature cells are treated than if the immature cells are subjected to irradiations. Investigation of the sterility produced in flies treated as adults and in those irradiated while in the larval stages aids materially in explaining the increased rate of induction in adult males as compared with the transmutation frequency in immature cells of the male. The percentage of sterility among male flies treated as larvae is found to be 52 ± 1.7 percent when males are X-rayed as 71–72 hour larvae, and 55 ± 1.5 percent when males are irradiated at the larval age of 35–36 hours. Obviously, the sterility of the two groups treated as larvae is very high compared with 2 ± 1.7 percent of sterility observed among treated adults. Since there is good reason to believe that sterility is associated with physical variation (unpublished data) and is very likely linked with gene mutation or chromosomal aberration, it seems plausible to con-

clude that much of the gonadic tissue of sterile individuals is composed of mutant cells. It is also probable that the low rate observed when early sperm cells were treated is partially due to a differential reduplication of non-mutated as compared to mutated gonial cells. If the reduplication of non-mutant and mutant cells had proceeded at the same rates and if it had been possible for the sterile individuals to reproduce, many more mutant cells would have been detected among offspring of males treated as larvae, raising the mutation frequencies to the same (or perhaps a higher) level as that observed when adult males were irradiated. If the mutation rate observed for females, treated as adults, is compared with that induced by irradiating either larval stage, no importance can be attached to the slight difference in the rates. This lack of variation is satisfactorily explained by the fact that female germ cells contain two X chromosomes that protect them against differential reduplication. The differences existing between the mutation rate induced by raying adult males and the rate observed when females are treated as 71–72 hour, or 35–36 hour larvae are just within the bounds of significance. If sterility had been less frequent in these larval groups the induced mutation rates would probably have been approximately equal in value to that obtained by treating mature sperm cells. Hence, one can safely conclude that the genic material of a cell is equally susceptible to the transmuting effects of irradiations irrespective of the sex, or stage of development at the time of treatment, but associated factors render the detection of all mutations impossible when immature germ cells are irradiated. It is interesting to note that the results upon which this conclusion is based are in conflict with the findings of MULLER (1930) who reported that "the rate of induction in mature sperm is higher than that in adult females."

As regards the chromatin content, the question of the morphology of the chromosome in the mature germ cell is of extreme interest. KAUFMANN (1925) describes the chromosome of *Tradescantia pilosa* in somatic metaphase, insofar as the chromatic material is concerned, as a four-parted structure, a pair of parallel halves each containing duality of elements. NEBEL (1932), working with the same form, observed four chromonemata in each dyad of the meiotic metaphase. Our knowledge of the subsequent behavior, in the meiotic divisions, leaves doubt as to the number of chromonemata, or duplicate genes contained by the chromosomes of the mature sex cells. If there is no reduplication of chromonemata following the maturation divisions, the chromosomes of the mature sex cells of Tradescantia should contain two chromatin threads, or a duplicate set of genes. In case reduplication is the rule, four chromonemata, or the equivalent genic complex should be present in each chromosome of the mature sex cells. The cytological findings in Tradescantia stimulate interest as to

the conditions existing in Drosophila. KAUFMANN, in 1931, was unable to follow the chromonemata of Drosophila chromosomes through the various stages of cell division, but observed the presence of more than one chromatin thread. PATTERSON, in 1933, working on the production of mosaic flies by breaking the X chromosome of Drosophila, concludes that the X chromosome of the sperm cell is split in about one out of every seven sperms, and that the chromosome of the egg is a single thread at the time of treatment.

The results tabulated in table 3 offer a basis for the solution of the problem as to the genic complex in the X chromosome of Drosophila. The first line of table 3 records the data on mutations obtained from treated adult males. It has been pointed out that the various mutations, or variations, are divided into four distinct groups. The criteria for the classification have been explained. According to the data from treated adult males, ninety-eight "radical" mutations were detected. The sterile individuals (59) constitute approximately 60 percent of the total, while thirty-nine, or about 40 percent of the flies bred true. Among the "partial" mutations, 103 individuals were one-half mutant, of which twenty-one were sterile, eleven bred as mutant and seventy-one reproduced the normal type. Four quarter-mutants and eight "minute" mutations are listed. The number of "partial" mutations totals 115, thirteen of which bred true. This is interpreted to mean that two sets of genes are present in the X chromosome of the sperm at the time of treatment. Since nothing definite is known concerning the way irradiations affect the gene, one cannot predict the ratio of "radical" to "partial" mutants that should be obtained if the chromosome contains two chromonemata, or a duplicate set of genes. The ratio depends entirely upon the probability of single and simultaneous mutations at a given locus. However, it is impossible to account for the large proportion of individuals expressing the variations in one-half the tissues on the basis of a single thread of genes. Even the small number of flies that appear phenotypically "partial" and breed true, either as mosaics or as "radical" mutations, cannot be accounted for by such a simple assumption. Further, if more than duplicate genes exist in the chromosome of the sperm cells, many mosaics would occur with one-fourth, or less, of the tissues mutant. Actually twelve such cases were obtained from males treated as adults. Therefore, no alternative is at hand. An explanation of the results rests upon the assumption of a chromosome, the chromatic element of which consists of two chromonemata, or the genic equivalent. Such an assumption offers a complete explanation of the phenotypically half and half mosaics. It also lends understanding to the "radical" aberrant cases if we admit the possibility of transmutations occurring simultaneously in allelic genes,—the simultaneous mutation of two genes occu-

pying allelic loci in the chromonemata. It is obvious that complete, or "radical" mutations occur, and since it is improbable that the chromatin material of the sperm cell assumes a definite configuration in one cell and an entirely different condition in another, there is no choice but to concede the production of simultaneous mutations of allelic elements. Only the twelve mosaic individuals having one-fourth, or less, of the tissues mutant remain to be explained. These offer no difficulty when we recall that HUETTNER (1926) reports irregular cleavage as occurring frequently in Drosophila, and that STURTEVANT (1929) found cleavage to be indeterminate in gynandromorphs of *Drosophila simulans*. The individuals under consideration probably started development as half and half mosaics, in so far as genetic content is concerned, and may be accounted for on the basis of indeterminate cleavage which permits unequal distribution of mutant cells as compared to the non-mutant cells. Therefore, all things considered, the premise that the mature sperm cells bear chromosomes composed of duplicate genic threads supplies the only condition that fulfills the genetic requirement.

If adult females are treated the chromosomes of the egg cells lie in the equatorial plate of the first meiotic metaphase at the time of irradiation. Obviously, if we postulate one set of genes per chromosome, the appearance of mosaic individuals becomes, *a priori*, an impossibility. Likewise, if we assume each chromosome of this stage to consist of two genic strands, no mosaic individuals can be produced by irradiation. If four chromonemata, or the genetic equivalent, are present in each dyad of the first meiotic metaphase plate, the genetic result, with respect to the production of "radical" mutations and mosaic flies should parallel the result obtained by treating mature sperm. As the data of table 3 show, 77 "radical" mutations were obtained from females treated as adults, 55 of which were sterile and 22 bred true. Approximately an equal number of one-half mutant flies are recorded. A total of 92 such individuals contain 20 sterile flies, 12 breeding as mutants and 60 that produced normal offspring. As in the case of treated males, comparatively few mosaic flies are recorded that show one-fourth, or less, of the body mutant. Nine such cases are present, one of which bred as a mutation. This result is almost identical with that obtained by treating the mature males with irradiation, and therefore, fulfills perfectly the requirements of the assumption that the dyad of the first meiotic metaphase is composed of four chromonemata.

It seems that no conflict exists between the concept of duplication of genes, or the postulated number of chromonemata, and the inferences of PATTERSON'S findings on chromosome breakage. According to the view of the writer, the two results are the expressions of entirely different phenomena. Chromosome breakage is a phenomenon of the matrix. It involves

the physical property of separating the material bonds of the matric substance as well as severing the inter-connections of the genes. The question of chromosome structure as revealed by breakage is apparently a question of the condition of the matric material. Chromosomes in which the matrix is split may be broken in only one arm of the divided chromosome, behaving subsequently as if two chromonemata are present. A break of an undivided matrix results in the severing of all the chromonemata, the behavior in subsequent inheritance suggesting a single chromatin thread. Two, four, or more genic threads may be present in the chromosome and yet appear as one or two depending upon the condition of the matrix at the time the break occurred. One might expect the matrix to be unsplit in some cells and split in others, or vesiculated, which, if broken through one hemisphere of the vesicle, would react as if the matrix were divided, but it seems improbable that the chromatic elements of the cells would present varied pictures. Hence, it seems probable that breakage is a phenomenon of the matrix, and mutation a function of the genes which lie in the chromonemata. This being the case the data obtained by breakage and that derived from mutations compel no conflicting conclusions.

A word as to the behavior of mosaic flies in breeding tests is necessary to explain the preponderance of phenotypic "partials" that produce only normal offspring. The behavior of the mosaic individuals obtained by treating adult males is typical and serves as an example for discussion. In view of the predictions of HUETTNER (1926) concerning sex mosaics and of the findings of DOBZHANSKY (1930) in gynandromorphs, one would expect the gonads of 50 percent of the so-called bilaterial mosaic individuals to be composed of one type of tissue,—either mutant or non-mutant. Therefore, since variation is frequently accompanied by sterility, the sterile half-mutants plus the half-mosaics breeding as "radical" mutations, should constitute about one-fourth the total population of the group tabulated as one-half mutant. Actually, 21 of the 103 individuals of this type are sterile and 6 prove to contain only mutant tissue in the gonads. The total of 21 individuals is slightly more than one-fourth the total number of males classified as one-half mutant. Five individuals of the class prove to contain both mutant and non-mutant cells in the testes, whereas, approximately 50 individuals should produce both normal and mutant offspring, and about 25 should give normal flies. Part of the 45 males that should have produced both normal and mutant ofispring due to a mixture of tissues in the reproductive glands, but which bred as normal, were probably carriers of chromosomal abnormalities which were lethal in hyperploid and hypoploid offspring. Offspring develop from the normal germ cells but the abnormal cells are incapable of forming viable zygotes. The behavior of a part of the irregular individuals may be attributed to dominant

lethals carried by a comparatively small number of cells of the mosaic fly. Normal tissue renders the mosaic viable but the mutants are lethal to the offspring receiving them. Recessive lethal mutations associated with visibles of the X chromosome will account for a portion of the irregular individuals. Considering all the possibilities, the results of breeding tests compare favorably with the expectations.

I wish to express appreciation to Professor J. T. PATTERSON for the suggesting of the problem and for his helpful advice concerning the experimental work. I also express thanks to Professor H. J. MULLER for suggestions of method.

CONCLUSIONS

1. Irradiations produce mutations in *Drosophila melanogaster* irrespective of the developmental stage treated, or of the sex of the individuals to which the X-rays are administered. There is no difference in the mutation frequency induced by irradiating females and that induced by the treatment of males in the same stage of development. The sex of the cell exerts no influence upon the mutability of the gene.

2. The detectable mutation frequency obtained by treating mature males, or mature females with X-rays is significantly higher than that observed when certain groups of males, or certain groups of females are treated as larvae. All factors considered, the gene is probably as mutable in one stage of development as in any other stage, but all mutations cannot be detected when produced in certain stages of the germ cells.

3. The chromosomes of mature sperm cells contain two complete sets of genes as chromonemata, or potential chromonemata. It appears that each monad of the reductional metaphase of the egg is composed of two complete genic complexes in the form of chromonema threads.

LITERATURE CITED

BUCHHOLZ, J. T. and BLAKESLEE, A. F., 1928 Radium experiments with Datura. I. The identification and transmission of lethals of pollen-tube growth in F_1's from radium treated parents. (abstr.) Anat. Rec. **41**: 98.

1928 Radium experiments with Datura. V. Effects of radium on pollen-tube growth rate, and the seed output following treatment. (abstr.) Anat. Rec. **41**: 101.

DOBZHANSKY, T., 1930 Interaction between female and male parts in gynandromorphs of *Drosophila similans*. Wilhelm Roux' Arch. f. Entwick. d. Org. **123**: 719–745.

GOODSPEED, T. H., 1929 The effects of X-rays and radium on species of the genus Nicotiana. J. Hered. **20**:243–259.

HANSON, F. B., 1929 The effects of X-rays on productivity and the sex ratio in *Drosophila melanogaster*. Am. Nat. **62**: 352–362.

HANSON, F. B. and WINKLEMAN, E., 1929 Visible mutations following radium irradiation in Drosophila. J. Hered. **20**: 277–286.

HARRIS, B. B., 1929 The effects of aging on X-rayed males upon mutation frequency in Drosophila. J. Hered. **20**: 299–302.

HUETTNER, A. F., 1923 The origin of the germ cells in *Drosophila melanogaster*. J. Morph. **37**: 285–423.

1924 Maturation and fertilization in *Drosophila melanogaster*. J. Morph. **39**: 249–256.

1926 Irregularities in the early development of the *Drosophila melanogaster* egg. Zeit. F. Zellfor. u. Mikro. Anat. **4**: 599–610.

KAUFMANN, B. P., 1925 Chromosome structure and its relation to the chromosome cycle. Amer. J. Bot. **13**: 59–80.

1931 Chromonemata in somatic and meiotic mitoses. Amer. Nat. **65**: 280–283.

1931 Chromosome structure in Drosophila. Amer. Nat. **65**: 555–557.

MOORE, W. G., 1932 The effects of X-rays on fertility in *Drosophila melanogaster* treated at different stages in development. Biol. Bull. **62**: 294–305.

MULLER, H. J., 1927 The problem of genic modification. Z.I.A.V. **1**: 234–260.

1928 The production of mutations by X-rays. Proc. Nat. Acad. Sci. Washington **14**: 714–726.

1929 Heritable variations, their production by X-rays and their relation to evolution. Smith. Report, 345–362.

1929 The gene as a basis of life. Proc. Int. Congress Plant Sci. **1**: 897–921.

1930 Radiation and genetics. Amer. Nat. **64**: 220–251.

1930 Types of visible mutations induced by X-rays in Drosophila. J. Genet. **22**: 299–334.

NEBEL, S. R., 1932 Chromosome structure in Tradescantia. Zeit. f. Zellfor. u. Mikro. Anat. **16**: 251-304.

OLIVER, C. P., 1930 Effects of varying the duration of X-ray treatment upon the frequency of mutations. Science **71**: 44–46.

1932 An analysis of the effect of varying the duration of X-ray treatment upon the frequency of mutations. Z.I.A.V. **61**: 447–488.

PATTERSON, J. T., 1928 The effects of X-rays in producing mutations in the somatic cells of *Drosophila melanogaster*. Science **68**: 41–43.

1929 X-rays and somatic mutations. J. Hered. **20**: 261–267.

1929 The production of mutations in somatic cells of *Drosophila melanogaster* by means of X-rays. J. Exp. Zool. **53**: 327–372.

1929 Somatic segregation produced by X-rays in *Drosophila melanogaster*. Proc. Nat. Acad. Sci. Washington **16**: 109–111.

1930 Proof that the entire chromosome is not eliminated in the production of somatic variations by X-rays in Drosophila. Genetics **15**: 141–149.

1933 The mechanism of mosaic formation in Drosophila. Genetics **18**: 32–52.

PATTERSON, J. T. and PAINTER, T. S., 1931 A mottled-eyed Drosophila. Science **73**: 530–531.

STADLER, L. J., 1928a Genetic effects of X-rays in maize and barley. Anat. Rec. **37**: 176.

1928b Genetic effects of X-rays in maize. Proc. Nat. Acad. Sci. Washington **14**: 69–75.

1928c Mutations in barley induced by X-rays and Radium. Science **68**: 186.

STANCATI, M. F., 1932 Production of dominant lethal genetic effects by radiation of sperm in Habrobracon. Science **76**: 197–198.

STURTEVANT, A. H., 1929 The claret mutant type of Drosophila. A study of chromosome elimination and of cell-lineage Zeit. f. Wissenach Zool. **135**: 323–356.

WHITING, P. W., 1929 X-rays and parasitic wasps. J. Hered. **20**: 269–276.

CROSSING OVER INDUCED BY X-RAYS IN DROSOPHILA MALES

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INTRODUCTION

It has long been known that crossing over does not take place in the male of Drosophila. In attempts to explain this fact several different suggestions have been made, among them the failure of synapsis to occur in the male germ cell. Dr. BESSIE LEAGUE (unpublished paper) has since shown cytologically that synapsis does occur in the male, but the process takes place rapidly and is easily overlooked. Although generally crossing over does not take place in the heterozygous sex, yet it does occur in both sexes in several different species, notably in the locust, a crustacean, and the mouse. More than a year ago the senior author obtained, in a preliminary experiment, evidence that crossing over could be induced in the male germ cell of *Drosophila melanogaster* by means of X-radiation. Since then the experiments have been extended, and it is the object of this paper to give the evidence upon which this conclusion was based. The junior author carried out all of the linkage tests on the stocks thus derived from induced crossing over. We wish to acknowledge our indebtedness to Mr. WILSON STONE for valuable help in connection with the linkage studies.

In order to produce an exchange of segments between homologous chromosomes, it is necessary to treat male larval stages which are known to contain immature germ cells. If the larvae are made heterozygous for a series of recessive mutant genes in any one of the pairs of autosomes, an induced exchange of segments between the homologues of this pair could be detected among the offspring arising from the backcross. In case the exchange involved segments of equal length of the two homologous elements, and produced viable zygotes, the result would be equivalent to *induced crossing over*.

The long III chromosome was chosen for the test. The experiments were initiated by crossing normal females to rucuca/Moiré-claret males, and then mating individually the F_1 non-Moiré males from the treated larvae to rucuca/Moiré females. The rucuca stock carries eight recessive genes in the III chromosome (*ru h th st cu sr e^s ca*). On account of the poor viability of homozygous flies, the rucuca stock is carried in heterozygous form, balanced to a III chromosome, known as Moiré, which has a gene rearrangement that prevents crossing over except at the extreme right end,

or the region of claret, where a slight amount of crossing over occurs. Flies carrying the Moiré chromosome, except when homozygous for claret, exhibit the dominant variegated eye-color (GLASS 1932), and can thus be distinguished from those not having this chromosome.

A few normal crossovers (between sooty and claret) will occur in the females used in the backcross, giving claret and *ru h th st cu sr e*^{s}. The F_2 claret flies can readily be tested to determine whether the presence of claret has been the result of a normal crossover or of an induced change. If a claret fly, when mated to wild-type, gives Moiré individuals among its offspring, this would indicate that it arose from a normal crossover, because in that event the fly must have received from its mother a III chromosome carrying the gene for variegated eye-color, which lies in the left arm of the Moiré chromosome. If, however, the tested F_2 claret fly yielded no Moiré progeny we would then know that the presence of claret was due to some effect produced by the X-radiation—an induced crossover, a point mutation, or, possibly, a deficiency. We have tested all of the F_2 claret flies obtained in the control and experimental series by this method.

In conducting the experiments, the flies were allowed to lay eggs for twelve hours on food contained in paper spoons placed in one by four inch culture vials. At the end of each twelve-hour period the spoons were removed and new ones with fresh food were introduced. The cultures were then allowed to develop until the larvae had reached a stage at which it was desired to give the X-ray treatments. Each culture was divided, one half being treated and the other half being used as a control. The flies and developing cultures were kept in an air-cooled room in which a temperature of 76 degrees Fahrenheit was maintained.

EXPERIMENTAL RESULTS

The exposures were given at four different stages of development namely, 24 to 36 hours, 40 to 52 hours, 60 to 72 hours, and 72 to 84 hours. All treated cultures were given a dosage of 1325 r units. We have included the results obtained from all the experiments and their controls in table 1. Only the F_2 flies in which the induced variation could be detected are listed in the table. The Moiré flies were disregarded.

The figures show that twenty-three classes of flies were obtained, and that the total number of flies in the experimental series was 8371 while the control series gave 8329 flies. The first and second classes constitute the expected non-variant wild-type and rucuca flies, respectively. The third class includes the normal crossover arising from the rucuca-Moiré females used in the backcross, while the fourth class includes the reciprocal crossovers arising from the same type of female. The flies belonging to this class were determined by testing all claret flies to wild-type, as was suggested

above. The remaining classes, fifth to twenty-third, include all other variants, and, with one exception (one *ru h th st* fly of the control series), represent variations induced by the X-radiation. The number of variants for each of the four stages at which the exposures were given is as follows: five for the I-stage, twenty-six for the II-stage, twenty-seven for the III-stage, and nineteen for the IV stage. This gives percentages of 0.27, 1.65, 1.03, and 0.81, respectively, for the four stages.

The highest rate occurred in larvae treated at forty to fifty-two hours, while the lowest occurred in larvae treated at twenty-four to thirty-six hours, the other two stages showing intermediate rates. We take this to mean that X-radiation is most effective in cells that are in the growth stages of the germ-cell cycle. Dr. LEAGUE has shown that at twenty-four hours old the larval testis contains chiefly spermatogonia, with only slight indications of growth stages. In the testes of thirty-six hour larvae growth stages are more noticeable. In older larvae mid-growth stages are found, and, finally, in larvae as much as eighty-four hours old well-developed primary spermatocytes are present. We thus see that the highest rate produced by the X-rays takes place in larvae containing the largest number of germ cells in the early growth stage. This corresponds to the stage in the female germ cell in which normal crossing over is supposed to take place (PLOUGH 1917). The fact that a few variants were produced in the youngest treated stages (twenty-four to thirty-six hours) probably means nothing more than that such variations were induced in the oldest larvae in the culture, that is, in larvae that had reached, or nearly reached, the thirty-six hour stage. The continuous development of spermatozoa in Drosophila will account for the fact that treating larvae that had passed the peak of early growth stages, results in the production of fewer variants of the type with which this paper is concerned.

Genetic studies show that many of the induced variations listed in the table were the result of an exchange of segments between the homologues of the III chromosome. The evidence further shows that in some of the cases the two chromosomes must have been matched gene by gene at the time the exchange was induced. In this sense the exchange so closely simulated normal crossing over in the female germ cell as to warrant its designation by the term induced crossing over.

Table 1 likewise shows that the majority of crossovers occurred between the loci of *st* and *cu* and in the two adjacent regions. This is in accordance with experimental data indicating that the middle of the III chromosome, or the spindle fiber region, is most susceptible to X-ray treatment (MULLER 1925). It also corresponds to the region most susceptible to heat (PLOUGH 1921), and to triploidy (REDFIELD 1930).

Of the 77 variants caused by X-radiation, 18 were either sterile or died

before breeding tests were completed. Forty-six of the 59 fertile cases were bred long enough to determine that 37 of these were viable in homozygous form and 9 non-viable. The location of the lethal was determined in six cases, and will be discussed later. Seven of the non-viable cases represent variants that involve a single marked locus. These are similar to cases ob-

TABLE 1

CLASSES	AGE IN HOURS AT WHICH TREATMENTS WERE GIVEN				CONTROLS
	I 24 TO 36	II 40 TO 52	III 60 TO 72	IV 72 TO 74	
1. Wild-type	1386	1217	1906	1926	6321
2. rucuca	446	330	681	374	1993
3. *ru h th st cu sr e*^{s}	4	1	1	2	7
4. *ca* (normal crossovers)	10	0	7	3	7
5. *ca* (induced change)	2	1	8	1	0
6. *ru th st cu sr e*^{s} *ca*	0	0	1	0	0
7. *ru h th st sr e*^{s} *ca*	0	0	1	1	0
8. *ru*	0	1	1	2	0
9. *th*	0	0	1	0	0
10. *st*	0	1	1	0	0
11. *cu*	0	1	0	0	0
12. *sr*	0	1	0	0	0
13. *ru ca*	0	2	1	0	0
14. *th st*	0	0	0	1	0
15. *ru h*	0	1	0	2	0
16. *ru h th*	0	0	2	0	0
17. *ru h th st*	1	10	10	5	1
18. *st cu sr e*^{s} *ca*	0	0	0	1	0
19. *cu sr e*^{s} *ca*	0	3	1	2	0
20. *ru h th st cu*	1	0	0	1	0
21. *sr e*^{s} *ca*	1	3	0	3	0
22. *e*^{s} *ca*	0	1	0	0	0
23. *ru h th st cu sr*	0	1	0	0	0
Totals	1851	1574	2622	2324	8329

tained by X-raying the X chromosome, and like them, probably represent small deficiencies, except in cases where the lethal proved to be away from the marked region (PATTERSON 1932). It is significant that in the majority of cases from variants showing two or more of the mutant characters, flies homozygous for the affected region are viable. This shows that the induced variation was not the result of a deficiency caused by loss of a piece of the treated chromosome.

The stocks established from variant flies were kept in heterozygous form, balanced to *Mo ca* or *ru h Dcx*. Thirty-seven such stocks were developed. In case the variant fly was a male, a single stock was established; if the variant was a female, three lines of the same stock were established

from her sons. This was done as a precaution against the possibility that normal crossing over in the female might produce a son in which the affected region had been crossed out.

It is of the greatest importance to know whether or not the point, or points, at which the induced exchange of segments occurred involved a demonstrable chromosome abnormality (duplication or deficiency). This can be determined by crossover tests, which are described in the next section.

CROSSOVER TESTS

Of the thirty-seven stocks which were built up from variant flies, twenty were tested to determine if crossing over was normal. These stocks represented crossovers in each region of the rucuca chromosome. With the exception of region 6, each region was represented by two or more stocks. Since the object of the crossover experiments was to determine if the break in the chromosome which had been responsible for a variant fly was accompanied by any abnormality, crossovers in the backcross were counted only in the region of the break. However, a preliminary count was made in every stock to determine if crossing over occurred in each marked region.

The procedure in each case was the same. Stock virgins were crossed to males of a suitable composition to produce heterozygous females with the two mutants adjacent to the break in opposite chromosomes. For stocks having *ru* only, or *ru* and *ca*, "hes" (*h th st p cu sr e*^{s}) was used in the backcross. For the other cases special stocks were built up from males having two or more mutant genes adjacent to the break in the stock being tested. These males were crossover flies obtained from the control crossover experiment, and were balanced to *Mo ca*/rucuca, or *ru h Dcx*/rucuca. Because of an inversion, the Dichaete chromosome crosses over only occasionally in the *ru h* region, and even less in the region of *ca*. Like Moiré, homozygous Dichaete is lethal. Homozygous males and females of all stocks were used so far as possible to decrease the number of *Mo* and *D* flies, which had to be discarded. The phenotypically normal F_1 females were selected and kept 3 to 5 days to make sure all were virgin. They were then backcrossed to *Mo ca*/rucuca or *ru h Dcx*/rucuca males with ten pairs to a yeast bottle. At the end of six days the flies were transferred to a second yeast bottle. The *Mo* and *ru h Dcx* F_2 flies were discarded, and the remainder classified. The reciprocal classes of crossover flies were counted in separate columns in all cases. In approximately half the cases the reciprocal classes of noncrossovers were likewise counted separately. In a number of cases, the flies hatching during the second six-day period were kept separate from those of the first period to determine the difference in crossing over, if any.

Six of the stocks tested did not live homozygous. To locate the lethals, males with all possible combinations of mutant genes were selected during

the crossover experiments and crossed to *Mo ca*/rucuca or *ru h Dcx*/rucuca females. The males carrying the lethal chromosome over *Mo ca* or *ru h Dcx* were then backcrossed to females of the original stock, as will be described later in more detail.

For the control, wild females were crossed to *Mo ca*/rucuca males and the non *Mo* F_1 females backcrossed to *ru h Dcx*/rucuca males. To reduce the possibility of error in the *ca* region and to eliminate the Dichaete flies, an attempt was made to backcross the heterozygous females to homozygous rucuca males. No larvae were produced in this cross, even though a trial bottle of homozygous rucuca males and females showed larvae which, however, died on the fourth day.

When it became apparent that crossing over values in the middle of the chromosome were unusually low because of viability differences, a new control was set up with females having *ru h th st* in one chromosome and *cu sr* e^a in the other. The set up of this control was more nearly like that of the experiments. The F_1 females were crossed to *ru h Dcx*/rucuca as before. The results given in table 2 are from this control, with the exception of the e^a *ca* region, the data for which are from the first control. The crossing over percentages were essentially the same at the ends of the chromosome in both controls; in the middle, around *th*, *st* and *cu*, the second control gave a more accurate percentage of crossing over, while the intermediate regions varied only slightly.

Crossing over in region 1, ru-h

Region 1 was represented by three single crossover stocks, 513 and 1141, developed from males, and 1346 developed from a female; and by two double crossover stocks, both derived from females. All stocks were *ru*. Virgin females were crossed to "hes" males and the F_1 females back crossed to *Mo ca*/rucuca.

For stock 513, 841 crossover flies were obtained in a total of 3129. The percent of crossing over was 26.6, which is 0.6 percent more than the control. The variation is less than the error of difference, which is 0.75 percent. In stock 1141, the total number of flies was 3656. Of these, 879 were crossovers, giving a crossover percent of 24.0. The 2.0 percent variation from the control is less than three times the error of difference, which is 0.71 percent. The total number of flies obtained from stock 1346 was 3687. The crossovers numbered 923, giving a crossover percent of 25.0. The variation from the control is 1.0 percent, which is less than twice the 0.71 percent error of difference. These figures are the combined data for two separate stocks of 1346 derived from sons of the variant female. The total number of flies counted for stock 539 was 4784. The number of crossovers in region 1 was 1194, giving a crossover percent of 25.0. The variation from

the control is 1.0 percent, which is less than twice the 0.67 percent error of difference. The percent of crossing over for the two stocks of 539, which when combined gave the results shown here, differed only 0.1 percent from each other. For stock 588, 7628 flies were counted. Of these, 1893 were crossovers in region 1. The percent of crossing over is 24.8, which is 1.2 percent less than the control. This variation is less than twice the error of

TABLE 2

Crossing over in the different regions.

STOCK	REGION	TOTAL	CROSSOVERS	PERCENT	CONTROL PERCENT	S.D.	DIFFERENCE
513, *ru*	1, *ru-h*	3129	841	26.6		.75	0.6
1141 "	"	3656	879	24.0	26.0	.71	2.0
1346 "	"	3687	923	25.0		.715	1.0
1309, *ru h*	2, *h-th*	3339	547	16.4		.63	1.5
1239 "	"	2767	525	18.9	17.9	.68	1.0
294 "	"	2498	491	19.7		.699	1.8
94, *ru h th*	3, *th-st*	3434	17	0.496		.107	.116
127 "	"	2041	12	0.575	0.38	.130	.155
1182 *st cu sr e*^{s} *ca*	"	7456	32	0.428		.077	.048
578, *ru h th st*	4, *st-cu*	2937	103	3.51		.339	0.25
103 "	"	2479	151	6.10		.333	2.84
545 "	"	2624	87	3.31	3.26	.399	0.05
274, *cu sr e*^{s} *ca*	"	4351	200	4.60		.296	1.34
1388 "	"	6867	256	3.78		.270	0.52
521, *sr e*^{s} *ca*	5, *cu-sr*	1676	186	11.1		.630	0.94
1199 "	"	2988	356	11.9	10.2	.540	1.70
1449 *ru h th st cu*	"	2105	256	12.1		.600	1.94
283, *e*^{s} *ca*	6, *sr-e*^{s}	5096	436	8.6	9.16	.44	.56
539, *ru-ca*	1 and 7 *ru-h—e*^{s}*ca*	4784	R 1, 1194	25.0	26.0	.670	1.0
			R 7, 1648	34.4	33.6	.634	0.8
588 "	"	7628	R 1, 1893	24.8	26.0	.625	1.2
			R 7, 2617	34.2	33.6	.565	0.6

difference, which is 0.625 percent. This represents the combined data for the three stocks of 588 which were derived from sons of the original variant female and tested separately. Only one case, which gave a crossover percent of 23.1, differed significantly from the control, and even this case is no longer significant when the data for the three stocks of 588 are combined. Crossing over in region 1 is thus normal in all experimental stocks which were tested.

Crossing over in region 2, h-th

Region 2, between *h* and *th*, was represented by three stocks, one derived from a male and two from females. All stocks were *ru h*, and were crossed to *th st cu* males.

For stock 1309, 3339 flies were counted. The total number of crossovers was 547, giving a crossover percent of 16.4. This is 1.5 percent less than the control, but the variation is less than three times the error of difference, which is 0.63 percent. The total number of flies counted for stock 1239 was 2767. The crossover percent calculated from 525 crossover flies was 18.9. This is 1.0 percent higher than the control. The variation is less than twice the error of difference, which is 0.68 percent. These data are the combined results of two separate stocks derived from sons of the original variant female. For stock 294, 2498 flies were obtained. Of these, 491 were crossovers, giving a crossover percent of 19.7. This is 1.8 percent more than the control, but the variation is less than three times the 0.69 percent error of difference. Although the values for the stocks tested for region 2 differ considerably and are all higher than the standard, they do not vary significantly from each other nor from the control. It is possible that the original crossover from which each of these stocks was derived occurred at a different locus between *h* and *th*.

Crossing over in region 3, th-st

All stocks tested for region 3 were derived from females. Two, stocks 94 and 127, were *ru h th* and were crossed to *st cu* males. Stock 1182 was *st cu sr e*^{s} *ca* and was crossed to *h th* males. F_1 females of both classes were backcrossed to *ru h Dcx*/rucuca.

For stock 94, 3434 flies were counted. Of these, seventeen were crossovers giving a crossover percent of 0.496. This is 0.116 percent higher than the control, but the variation is less than twice the error of difference, which is 0.107 percent. For stock 127 the total number of flies was 2041. Twelve crossovers gave a crossover percent of 0.575, which is 0.155 percent higher than the control. The variation is less than twice the error of difference, which is 0.13 percent. The total number of flies obtained from stock 1182 was 7456. Thirty-two crossovers gave a crossover percent of 0.428, which is 0.048 percent higher than the control. This variation is less than the error of difference, which is 0.077 percent. The figures for stock 1182 represent the results obtained from three separate stocks derived from sons of the variant female. All stocks tested for crossing over in region 3 gave values higher than the control but none varied significantly.

Crossing over in Region 4, st-cu

Both contrary classes were represented in the stocks tested for region 4.

Three were *ru h th st*, and two were *cu sr e*^{s} *ca*. All except stock 274 were derived from females.

For stock 578, *ru h th st*, 2937 flies were counted. Of these, 103 were crossovers, giving a crossover percent of 3.51. The 0.25 percent variation from the control is less than the error of difference, which is 0.339 percent. The total number of flies counted for stock 103, *ru h th st*, was 2479. The percent of crossing over calculated from 151 crossover flies was 6.1, which is 2.84 percent more than the control. Since this is more than three times the error of difference, which is 0.333 percent, the variation is significant. For stock 545, *ru h th st*, 2624 flies were counted. Eighty-seven of these were crossovers, giving a crossover percent of 3.31. The 0.05 percent variation from the control is less than the error of difference, which is 0.399 percent. For stock 274, *cu sr e*^{s} *ca*, 4351 flies were counted. The number of crossovers obtained was 200, giving a crossover percent of 4.6. The variation from the control is 1.34 percent, which is more than three times the error of difference, which is 0.296 percent. A total of 6867 flies was counted for stock 1388, *cu sr e*^{s} *ca*. The number of crossovers was 256, giving a crossover percent of 3.78. The 0.52 percent actual difference from the control is less than twice the error of difference, which is 0.27 percent. These data represent the combined figures of three stocks of 1388. Two of the above cases proved to be significantly higher than the control. It is possible that these stocks, 103 and 274, were not derived from true crossovers but were the result of an exchange of segments which were not wholly homologous. Stock 103 is the only case in which crossing over for region 4 was as high as the standard. The 4.6 percent crossover value for stock 274 shows that crossing over in this stock is still subject to the influence which reduces crossing over in the middle of the rucuca chromosome.

Crossing over in region 5, cu-sr

Region 5 was represented by both contrary classes of a crossover between *cu* and *sr*. Stocks 521 and 1449 were derived from males and stock 1199 from a female. The *sr e*^{s} *ca* stocks were crossed to *st cu* males and the *ru h th st cu* stock to *sr e*^{s} males. The F_1 females of both classes were backcrossed to *ru h Dcx*/rucuca.

For stock 521, *sr e*^{s} *ca*, 1676 flies were counted. Of these, 186 were crossovers, giving a crossover percent of 11.1. This is 0.94 percent higher than the control. The variation is less than twice the error of difference, which is 0.63 percent. The total number of flies obtained from the two stocks of 1199 was 2988. Of these, 356 were crossovers, giving a crossover percent of 11.9. This is 1.7 percent higher than the control, and varies slightly more than three times the error of difference, which is 0.54 percent. For stock 1449, *ru h th st cu*, 2105 flies were counted. The number of crossovers was

256, giving a crossover percent of 12.14. This is 1.94 percent more than the control, and varies more than three times the error of difference, which is 0.6 percent.

All three cases had higher rates of crossing over than the control, the crossover percent of two of them proving to be significantly higher. Crossing over in region 5 of the control may be slightly reduced because of the same factor which reduces crossing over in regions 3 and 4. In this case the inhibiting effect may have been eliminated in these crossover stocks, thus permitting normal crossing over. Or, owing to the difficulty of distinguishing *sr*, *sr* e^s, or e^s alone in an unselected rucuca stock, the crossover values for regions 5 and 6 of the control may not represent the true crossover ratio between the two regions. It is possible, judging from the close agreement of the three experimental stocks with each other and with the standard, that the results of these experiments are more accurate than those of the control. On this assumption crossing over in these stocks may be considered as perfectly normal.

Crossing over in region 6, sr-e^s

Region 6 was represented by only one stock which was derived from a female. Homozygous e^s *ca* flies were crossed to *cu sr* males and the F_1 females backcrossed to *ru h Dcx*/rucuca. The total number of flies counted was 5096. The crossovers numbered 436, giving a crossover percent of 8.6. The variation from the control is 0.56 percent, which is less than twice the 0.44 percent error of difference. These data are the combined results obtained from three separate stocks derived from the original female. All stocks gave practically the same amount of crossing over. Although the percent of crossing over does not vary significantly from the control, it is higher and agrees with the standard. The sooty in this stock was very dark and non-crossovers and crossovers between *sr* and e^s were easily distinguished. For this reason, the experimental figures for region 6, as suggested above for region 5, may be less subject to personal error than the same region in the control.

Crossing over in region 7

Region 7 was represented by two double crossover stocks, both derived from female. Stock females, *ru-ca*, were crossed to "hes" males and the F_1 females backcrossed to *ru h Dcx*/rucuca. The data for crossing over between *ru* and *h* have been given above in the results for region 1.

In stock 539, 4784 flies were obtained. Of these, 1648 were crossovers in region 7. This gave a crossover percent of 34.4, which is 0.8 percent higher than the control. The variation is less than twice the error of difference which is 0.634 percent. For stock 588, 7628 flies were counted. The cross-

overs in region 7 numbered 2617, giving 34.2 percent of crossing over. This is 0.6 percent higher than the control. The variation is less than twice the error of difference, which is 0.565 percent.

The data for region 7 are the combined results of two stocks of 539, and of three stocks of 588. There was no appreciable difference in crossing over in any of the five stocks, which were tested separately. The percent of crossing over for all was slightly higher than the control, but not significantly so. The fact that crossing over in both region 1 and region 7 was normal is evidence that the stocks were derived from true crossovers.

Location of Lethals

Of the twenty stocks which were tested for linkage of characters, six failed to live in the homozygous state. The locus of the lethal was determined by breeding males obtained from the linkage experiments. Males produced by crossing over in one or more of the marked regions were crossed to *Mo ca*/rucuca or *ru h Dcx*/rucuca. F_1 males carrying the lethal chromosome over *Mo ca* or *ruh Dcx* were then backcrossed to females of the original stock. The appearance among the offspring of the backcross of phenotypically normal flies, or of flies showing one or more characters of the lethal-bearing stock, indicated that the lethal had been crossed out. The production of *Mo* or *D* flies exclusively showed that the lethal was still present.

Tests of crossover males obtained from stock 1141, *ru*, showed that flies carrying any part of the original stock chromosome except the region of *sr* e^s produced viable zygotes when backcrossed. The lethal is therefore to the right of *sr*. The lethal in stock 1346, *ru*, was located between *cu* and *sr* in both stocks derived from the variant female. The lethal in stock 294, *ru h*, was located between the loci of *h* and *th*, perhaps at the point of breakage or crossing over. The lethal in stock 94, *ru h th*, was located between *th* and *st*, likewise at or near the point of crossing over. The lethal in the three stocks of 588 was located at the right of *th* and left of *cu* in the region of *st*. It was therefore associated with neither point of crossing over. The lethal in stock 1449, *ru h th st cu*, proved to be in the region of claret. These tests show that in four cases the lethal was not at the point of crossing over in the chromosome which determined the composition of the variant fly.[1] The agreement of the location of the lethals in both stocks of 1346 and in the three stocks of 588 shows that the sons from which the stocks were established carried identical chromosomes and that crossing over data can well be combined.

[1] Even in the two cases where the lethal was associated with the point of breakage, normal crossing over in the experiments indicates that the lethal effect was not due to a pronounced chromosomal abnormality.

The unexpected variant fly found in the control is of interest, because the case seems to represent a normal crossover occurring in the male germ cell. This variant was a *ru h th st* female that appeared in one of the F_2 control cultures (table 1). Tests were made and it was found that stocks developed from her sons were viable in the homozygous condition, exactly like those from similar variants obtained in the experimental series. No stocks of the composition of this female were present in the laboratory at this time, and the care with which the experiments were conducted would exclude the possibility of a "slip" in technique. It must, therefore, represent a very rare case of normal crossing over in the male.

DISCUSSION

The important point with reference to the experimental results described above is how the X-radiation has brought about the evident exchange of segments between the homologous chromosomes. There are two possible ways by which this may have been accomplished: (1) It is conceivable that X-radiation in some way modifies the inhibitory mechanism which prevents crossing over in the male germ cell; or (2) the exchange of segments between the homologues is brought about in the same manner as those producing mutual translocations between non-homologous chromosomes when mature germ cells are treated.

In this connection, it may be well to point out that the work on segmental interchange in Oenothera and Datura (CLELAND 1923, BELLING and BLAKESLEE 1926, DARLINGTON 1929) and on induced translocations in Drosophila (MULLER 1930, STURTEVANT and DOBZHANSKY 1930, BOLEN 1931, GLASS 1932, OLIVER and VAN ATTA 1933) has indicated that most translocations consist of an exchange of segments between two non-homologous chromosomes. Most of the reported cases in Drosophila have been obtained by treating mature germ cells, which contain the haploid number of chromosomes. Any exchange of segments would, therefore, have to occur between non-homologous elements; there would be no opportunity for the exchange of segments to take place between homologous chromosomes. In treating the male larval stages the opportunity for an exchange to occur between homologues is present, and such exchanges might be of the same general nature as those occurring in the case of mutual translocations.

It has been found, however, that a large majority of translocations in Drosophila are lethal in the homozygous condition. Miss SARAH C. BEDICHEK of this laboratory has recently completed a study of translocations between the II and III chromosomes in stocks developed by Mr. WILSON STONE. She found that out of 120 tested stocks, only nineteen, or less than sixteen percent, were viable when homozygous for the transloca-

tion. The lethal character of so many of these translocations when homozygous is not fully understood, but in most cases it is in some way related to the break in the chromosomes.

In contrast to these conditions found in translocations, the induced crossover stocks show a relatively high percentage of viability in homozygous; about eighty percent. Furthermore, genetic studies showed that in only two out of the six tested stocks that were non-viable in homozygous, was the lethal factor located at or near the point of breakage. It is evident, therefore, that a large majority of the induced crossover cases represent a phenomenon entirely different from that underlying ordinary mutual translocations.

This conclusion is strongly supported by the facts brought out in the crossover tests made by the junior author. A large majority of the tested stocks showed normal crossing over in the regions where the induced exchange of segments occurred. With two exceptions, such variations from the control and standard crossover values as were observed could be explained on the basis of differential viability. The two exceptions (Nos. 103, 274) gave values significantly higher than those of the control, suggesting that they were the result of an exchange of segments in homologues that were not entirely matched. In no case was crossing over significantly lower for an affected region. All of the evidence shows that the majority of variants produced by X-radiation must have arisen through an exchange of segments while the homologous chromosomes were matched gene by gene, and this would be equivalent to normal crossing over.

Exactly how X-radiation brings about this effect is not known. But, if it is assumed that one effect of X-rays is to retard mitosis, a suggested explanation of induced crossing over in Drosophila males can be offered. Dr. LEAGUE's observations show that synapsis actually occurs during the growth period of the primary spermatocyte, but that it represents only a fleeting phase of the maturation process, too rapid, perhaps, to allow for crossing over to take place. It may well be that the retarding effect of X-radiation on this process furnishes the necessary opportunity for crossing over to occur.

SUMMARY

1. The paper describes a series of experiments designed to induce by X-radiation crossing over in Drosophila males. To accomplish this, male larvae, heterozygous for a series of eight mutant genes in the III chromosome, were X-rayed and crossed to females having this same type of chromosome. In all, seventy-seven variants that could be assigned to the effects of X-rays were obtained in the F_1 cultures out of 8371 flies. Only one such fly was found among 8329 control flies.

2. Genetic tests showed that a majority of these cases were due to an

exchange of segments between the homologous III chromosomes. Such tests further showed that eighty percent of the cases were viable in the homozygous condition. Crossover tests demonstrated that a majority of the cases gave approximately normal crossover values for the regions affected by the induced crossovers.

3. The conclusion is reached that X-radiation induces crossing over in the male when applied to immature germ cells which contain the diploid number of chromosomes. The effect is much more marked when treatment is given during the growth stages of the spermatocyte that correspond to the stages in the female germ cell when normal crossing over is supposed to occur.

LITERATURE CITED

BEADLE, G. W., 1932 A possible influence of the spindle fiber on crossing over in Drosophila. Proc. Nat. Acad. Sci., Washington **18**: 160–165.

BELLING, JOHN and BLAKESLEE, A. F., 1925 On the attachments of non-homologous chromosomes at the reduction division in certain 25-chromosome Daturas. Proc. Nat. Acad. Sci., Washington **12**: 7–11.

BOLEN, HOMER R., 1931 A mutual translocation involving the fourth and the X chromosomes of Drosophila. Amer. Nat. **65**: 417–422.

CLELAND, R. E., 1925 Chromosome arrangements during meiosis in certain Oenotheras. Amer. Nat. **57**: 562–566.

DARLINGTON, C. D., 1929 Ring formation in Oenothera and other genera. J. Genet. **20**: 345–363.

GLASS, H. B., 1932 A study of dominant mosaic eye color mutants in Drosophila. Proc. Sixth Int. Congress Genetics **2**: 62–63.

MULLER, H. J., 1925 Regionally differential effect of X-rays on crossing over in autosomes of Drosophila. Genetics **10**: 470–507.

1930 Oenothera-like linkage of chromosomes in Drosophila. J. Genet. **32**: 335–355.

OLIVER, C. P. and VAN ATTA, E. W., 1932 Genetic and cytological correlations of chromosomal aberrations of Drosophila. Proc. Sixth Int. Congress Genetics **2**: 145–147.

PATTERSON, J. T., 1932 Lethal mutation and deficiencies produced in the X chromosome of *Drosophila melanogaster* by X-radiation. Amer. Nat. **64**: 193–206.

PLOUGH, H. H., 1917 The effect of temperature on crossing over in Drosophila. J. Exp. Zool. **24**: 147–193.

1921 Further studies on the effect of temperature on crossing over. J. Exp. Zool. **32**: 187–201.

REDFIELD, HELEN, 1930 Crossing over in the third chromosomes of triploids of *Drosophila melanogaster*. Genetics **15**: 206–251.

STURTEVANT, A. H., and DOBZHANSKY, T., 1930 Reciprocal translocations in Drosophila and their bearing on Oenothera cytology and genetics. Proc. Nat. Acad. Sci., Washington **16**: 533–536.

AZYGOTIC RATIOS IN HABROBRACON*

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There are gradually accumulating in genetic literature, data on organisms of widely diverse groups which may be known as azygotes. Such organisms arise from cells, unfertilized eggs in animals (parthenogenesis) or spores in plants, resulting from complete reduction in meiosis. Since it is now well known that reduction may occur in part in the first, in part in the second maturation division, even as regards a single chromosome tetrad, it is clear that two divisions are necessary to completely reduce the nucleus. This reduced nucleus is usually thought of as haploid but such is not necessarily the case. In tetraploid plants diploid spores result, while triploid females of Drosophila produce a certain proportion of diploid or otherwise not completely reduced eggs. Aside from such cases as polyploidy in which two divisions are not sufficient to effect complete reduction, there is increasing evidence for "regulation" of chromosome number following haploid parthenogenesis. The organism developing from an unfertilized egg or spore that has undergone complete reduction may be called an azygote, regardless of whether it is haploid or diploid. If regulation occurs the diploid organism becomes homozygous. It cannot be heterozygous except as a result of mutation. Organisms originating from diploid parthenogenesis may retain more or less of the heterozygosis of their mother and are therefore not azygous.

Geneticists are interested in determining gametic (or spore) ratios. Azygotes from heterozygous mothers, if obtained in numbers large enough to minimize deviations due to sampling, approximate these ratios with the following limitations:

(1) Masking effects due to an environmental condition or to another genetic factor.

(2) Overlapping of one class with another.

(3) Viability differences caused by one or more of the pairs of allelomorphs under consideration.

(4) Disproportion in viability differences when various factors are combined.

(5) Lethal and semilethal factors linked with one or more of the genes in question.

* Part of the cost of the accompanying tables is paid by the Committee on Effects of Radiation on Living Organisms (NATIONAL RESEARCH COUNCIL).

Subject to the above limitations zygotic ratios resulting from the cross of the heterozygote to the recessive approximate gametic ratios except that (5) linked lethal or semilethal factors have comparatively little influence. If the recessive stock be bred true, there is relatively little chance that it will contain lethals similar to any that may be linked with the heterozygous factors being tested.

In the case of sex linkage, linked lethal genes are more likely to cause deviations among the offspring of the digametic sex. Even in male Drosophila, however, it is possible that the Y chromosome may have some protective influence. In species with X-O male or Z-O female, the diagametic individuals are comparable with azygotes as far as sex-linked factors are concerned.

In males of Habrobracon all chromosomes are subject to this lethal selection, lacking protection from dominant allelomorphs. Review and discussion of azygotic ratios in other forms will be deferred until after the presentation of data on azygotic ratios in Habrobracon.

The present paper reports summaries of data which have been accumulated in recent years, largely resulting from tests for linkage made by graduate students associated with the UNIVERSITY OF PITTSBURGH. The authors wish especially to mention the work of MILTON FRANKLIN STANCATI, NEITA C. BOSTIAN, C. H. BOSTIAN, PAUL R. DAVID, P. J. WALTER, HELEN CASHDOLLAR, GEORGE D. SNELL, MAGNHILD M. TORVIK, KATHRYN A. GILMORE, and RAYMOND J. GREB.

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TECHNIQUE AND MATERIALS

Crosses are made involving two or more mutant types and F_1 females are isolated and bred as virgins. Counts of the broods furnish the ratios of males here reported, as well as irregular types (mosaics, impaternate females) occurring infrequently and disregarded in the present summaries. When two mutant types are female sterile, the female heterozygous for one is crossed with the male of the other, in which case only half of the F_2 fraternities are of value. The mutant factors may be introduced from different parents (repulsion tests, $a \times b$) or the double mutant type may be crossed with wild-type (coupling tests $a.b \times +$). It is advisable to make these reverse crosses to check deviations due to viability differences, but thus far most tests have been of the repulsion type as this is the more con-

venient way to test new factors. No consistent differences have been found between F_2 fraternities resulting from reciprocal crosses (a ♀ × b ♂ and b ♀ × a ♂ in case of repulsion tests or + ♀ × $a.b$ ♂ and $a.b$ ♀ × + ♂ in case of coupling tests).

Tests of the following mutant factors are here reported: Bar eye; Kidney, *k* (eyes) and its allelomorphs, small, k^s, and extreme-small, k^e; Orange, *o* (eye) and its allelomorph, ivory, o^i; Defective, *d* (R_4 wing vein); Wrinkled, *w* (wings); Reduced, *r* (wings); Miniature, *m* (body); Fused, *f* (segments of tarsi and antennae); Cantaloup, *c* (eyes); Long, *l* (antennae and wings); Wavy, *wa* (wings); Broad, *br* (thorax); Eyeless, *el*; Shot-veins, *sv* (wings); Maroon, *ma* (eyes); Yellow, *Y* (antennae); Semilong, *sl* (antennae and wings); Tapering, *ta* (antennae); Narrow, *n* (wings); Spread, *sp* (wings); Vestigial, *v* (wings); Beaded, *b* (legs and wings); Twisted, *tw* (legs); Stumpy, *st* (legs); White, *wh* (eyes); Crescent, *cr* (eyes); Cut, *ct* (wings); Club, *cl* (tarsi and wings); Glass, *gl* (eyes and antennae); Indented, *in* (wings); Truncated, *td* (wings); Crepe-wings, *cw*; Strap, *sr* (wings); Gynoid, *gy* (antennae and abdominal sclerites); Extended-head, *eh* (prothorax); Honey, *ho* (body color); Pointed, *p* (wings); Aciform, *ac* (antennae); Extended-wings, *ew*.

A description of some of these mutant traits together with an account of the occurrence of the mutations has been reported by the senior author (WHITING 1932). Others are new and will be described elsewhere. The eye mutations, bar, eyeless, small and extreme-small, were found and reported by WILHELMINA F. DUNNING (1931).

TABLE 1

Percentages deviating below 50 percent with probability equal to .90 or to .99 that true value is no lower in population from which sample of a given size, n, is taken.

n	27	32	37	42	47	55	65	75	85	95	110	130
Percent (P .90)	35	37	37	38	39	40	40	41	41	42	42	43
Percent (P .99)	27	29	30	31	32	34	35	36	36	37	38	39
n	150	170	190	250	350	450	550	800	1500	2500		
Percent (P .90)	44	44	44	45	46	46	47	47	48	48		
Percent (P .99)	40	40	41	42	43	44	45	45	47	47		

TABLE 2

Azygotic (male) frequencies of various combinations of the following genes: k, o, d, w, r, m, f, c, l, wa, br, el, sv, ma, Y, sl, ta, n, sp, v, b, tw, st, wh, cr, ct, cl, gl, in, td, cw, sr, gy, Eh, ho, p, ac, ew.

COMBINATION a—b	F_1 FEMALE	F_1 MALES +	a	b	a.b
k—o	$k^s.o^i/o.d$	26	4	22	28
		41	7	23	27
	$k^s.o^i/w$	130	93	50	164
		83	78	66	112

TABLE 2 (*Continued*)

COMBINATION a—b	F_1 FEMALE	F_2 MALES +	a	b	a.b
	k.o^i/r.br	47	23	34	46
		68	20	31	57
		45	19	25	23
		37	8	16	22
	k.o^i.r/sv	43	14	39	45
		47	16	30	32
		30	15	36	26
		31	21	15	21
	k.o^i.r/ta	40	10	29	26
		44	14	27	14
		31	21	15	25
		26	17	17	22
	k^s.o^i/m	38	69	259	664
		455	237	67	14
	k^s.o^i/o.br	92	104	57	116
		57	67	49	75
	k^s.o^i/sv	104	47	53	72
		57	51	47	72
	k^s.o^i/ma	43	42	71	201
		87	104		
	k^s.o^i/Y	22	21	8	35
		33	9	9	18
	k^s.o^i/sl	80	73	25	102
		34	120	15	126
	k^s.o^i/ta	65	47	18	101
		58	47	33	75
	k^s.o^i/ta	37	32	35	54
		62	35	25	52
	k^s.o^i/o.tw	69	42	44	69
		74	40	57	67
	k^s.o^i/st	9	7	6	8
		17	5	12	6
	k^s.o^i/cl	98	55	66	111
		26	15	15	32
	k^s.o^i.gl/+	15	8	13	23
		10	6	10	12
	k^s.o^i/Eh	171	69	111	191
		98	49	54	112

TABLE 2 (*Continued*)

COMBINATION a–b	F_1 FEMALE	F_2 MALES +	a	b	a.b
	$k^e.o^i/ac$	73	20	36	57
		58	19	31	53
$k-d$	k^e/d	48	32	64	34
$k-w$	k^e/w	180	257	149	190
$k-r$	$k.r/c.l$	52	5	74	13
		6	0	1	0
		13	1	4	0
		52	2	50	5
	k^e/r	205	235	186	223
	k/r	151	111	152	107
	$k.r/+$	715	309	683	301
$k-m$	k^e/m	297	733	522	251
$k-f$	k^e/f	497	515	379	425
$k-c$	k/c	329	38	266	15
$k-l$	k/l	133	18	119	8
$k-wa$	k^e/wa	188	197	166	136
$k-br$	k/br	180	146	123	72
	k^e/br	149	220	106	142
$k-el$	k^e/el	377	421	609	
	k^e/el	609	503	659	289
$k-sv$	k/sv	159	107	112	83
	k^e/sv	157	119	104	123
$k-ma$	k^e/ma	43	42	87	104
$k-Y$	k^e/Y	30	56	42	27
$k-sl$	k^e/sl	105	49	175	246
$k-ta$	k/ta	140	64	89	85
	k^e/ta	83	148	91	122
	k^e/ta	84	114	105	101
$k-v$	k^e/v	286	227	233	144
$k-tw$	k^e/tw	113	111	131	107
$k-st$	k^e/st	15	15	29	11
$k-wh$	k^e/wh	145	124	116	125
$k-cr$	k^e/cr	115	63	99	36
$k-ct$	k^e/ct	112	114	109	113
$k-cl$	k^e/cl	164	166	41	47
$k-gl$	$k^e.gl/+$	28	20	31	18
$k-cw$	k^e/cw	250	129	102	77
$k-gy$	k^e/gy	134	151	164	176
$k-eh$	k^e/eh	282	260	152	171
$k-ac$	k^e/ac	109	77	89	72
$o-d$	$o.d.r/+$	537	541	528	542
		976	1003		
	$o.d/wa$	115	92	94	101
		74	52	76	76
	$o.d/el$	52	53	75	70
		47	37	39	50

TABLE 2 (*Continued*)

COMBINATION a–b	F1 FEMALE	F2 MALES +	a	b	a.b
	o^{s}.*d*/*ma*	69	119	75	158
		62		75	
	o.d/*sl.wh*	64	63	71	66
		75	92	48	37
		103		128	
		139		100	
	o.d/*ta*	73	78	62	77
		89	77	64	51
	o.d/*n*	132	113	117	121
		72	72	98	74
	o.d/*v*	116	146	101	105
		199	182		
	o^{s}.*d*/*st*	86	102	73	67
		95	88	73	52
	o.d/+	3446	3486	3038	3004
	o^{s}.*d*/+	665	667	598	598
	o.d/*o*^{s}	30	50	48	50
o–*w*	*o.w*/*Y*	73	83	46	46
		48	47	36	49
	o.w/*n*	33	21	15	25
		15	12	14	13
	o.w/*ho*	91	79	73	77
		82	87	71	62
	o.w/+	1520	1391	1256	1327
	o/*w*	629	717	596	605
	o^{s}/*o.w*	128	130	134	130
	o^{s}/*w*	223	214	161	178
o–*r*	*o*^{s}.*r*/*m*	35	381	239	376
		217	25		
	pupae	141	10		
	o.r/*wa*	89	105	93	95
		91	101	66	107
	o^{s}.*r*/*el*	57	58	73	52
		29	44	22	24
	o^{s}.*r.Y*/+	37	32	24	19
		41	25	20	35
	o.r/*sl*	100	116	101	123
		99	117	109	124

TABLE 2 (*Continued*)

COMBINATION a—b	F_1 FEMALE	F_2 MALES +	a	b	a.b
	$o^i.r/n$	42	29	37	49
		25	14	25	12
	$o.r/sp$	133	126	137	154
		90	110	82	96
	$o^i.r/o.b$	31	45	31	28
		20	25		
	$o.r/+$	1788	1871	1656	1820
	$o^i.r/+$	821	977	789	871
	o/r	177	166	195	202
	o^i/r	292	303	312	314
	$o.r/o^i$	76	95	127	113
$o-m$	$o.m/c.l$	652	71	41	335
		39	6	1	29
		62	18	3	10
		766	90	74	149
	$o.m/+$	390	71	25	149
	pupae			15	45
	$o^i.m/+$	169	22	18	74
	pupae				2
	o/m	180	1528	738	82
	pupae			128	23
	o^i/m	153	1341	924	108
	pupae			145	10
	$o.m/o^i$	73	588	250	31
	pupae			14	3
$o-f$	$o(o^i)/f$	447	450	330	315
$o-c$	$o.c/el$	70	67	78	79
		72	171		
$o-l$	$o^i.l/Y$	58	60	45	57
		56	53	48	60
	$o^i.l/+$	206	191	177	208
	o^i/l	130	134	109	141
	$o^i.l/o$	80	85	88	85
$o-wa$	$o.ct/wa$	70	78	56	43
		38	52	16	27
	o/wa	499	523	379	406

TABLE 2 (*Continued*)

COMBINATION a–b	F_1 FEMALE	F_2 MALES +	a	b	a.b
o—br	*o^i.st/br*	97	96	81	86
		92	105	101	105
	o^i/br	543	542	415	401
	o.br/o^i	196	173	124	124
o—el	*o.el/sp*	100	99	60	
		84	94	22	
	o^i.ct/el	72	66	51	
		47	44	39	
	o.cw/el	61	47	23	
		49	46	85	
	o.el/+	184	193	82	
	o(o^i)/el	1136	1071	343	346
	o?			441	
o—sv	*o^i.st/sv*	36	38	27	13
		22	26	22	17
	o.cw/sv	234	204	63	62
		127	127	55	70
	o^i.sv.st/ho	26	19	28	25
		19	23	34	15
		30	20	26	13
		12	7	24	28
	o/sv	361	331	118	132
	o^i/sv	329	335	254	247
	o^i.sv/+	87	69	112	81
o—ma	*o^i/ma*	144	277	137	
	o^i(k^s)/ma	85	272	191	
	o/ma	167	157	96	98
o—Y	*o/Y*	238	209	205	174
	o^i/Y	205	212	187	170
	o^i.Y/+	208	175	145	135
o—sl	*o/sl*	336	368	331	370
	o^i/sl	153	127	154	141
o—ta	*o.sp/ta*	195	210	235	174
		80	48	61	77
	o^i.ct/ta	41	36	24	28
		26	29	21	35
	o.cw/ta	133	121	104	107
		59	61	42	51

TABLE 2 (*Continued*)

COMBINATION a–b	F_1 FEMALE	F_2 MALES +	a	b	ab
	o/ta	602	595	595	537
	o^i/ta	248	273	247	248
o–n	*o/n*	297	280	199	171
	o^i/n	453	484	266	179
o–sp	*o.sp/+*	430	384	151	125
	o/sp	370	379	256	300
o–v	*o/v*	217	251	199	182
o–b	*o^i.b/+*	138	133	25	33
	o^i/b	279	263	84	96
	o.b/o^i	62	73	20	25
o–tw	*o.tw/+*	318	324	180	157
	o.tw/o^i	111	113	114	124
o–st	*o/st.sr*	87	85	77	85
		91	78	99	101
	o/st	178	163	176	186
	o^i.st/+	351	310	326	326
	o^i/st	175	183	190	158
o–wh	*o/wh*	258	258	470	
o–ct	*o^i.ct/gl*	59	58	70	57
		79	60	67	50
	o(o^i).ct/+	486	453	361	367
o–cl	*o^i/cl*	153	177	41	47
o–gl	*o.gl/+*	128	117	109	96
	o^i.gl/+	23	36	16	22
	o^i/gl	129	115	146	110
o–in	*o/in*	128	137	98	122
o–td	*o/td*	134	147	133	140
o–cw	*o.cw/+*	595	543	332	325
o–sr	*o/sr*	164	170	190	179
o–gy	*o/gy*	138	136	118	159
o–Eh	*o^i/eh*	240	302	147	176
o–ho	*o/ho*	164	156	153	149
	o^i/ho	107	82	92	68
o–ac	*o^i/ac*	93	93	77	84
o–ew	*o^i/ew*	95	84	68	69
d–w	*d/w*	736	813	161	133
	d?			1028	
d–r	*d.r/+*	1078	1070	1979	
	d/r	335	260	42	584
d–f	*d.f/+*	46	41	29	26
	d/f	205	147	106	109
d–c	*d/c.l*	37	85	10	5
		6	4	84	26

TABLE 2 (*Continued*)

COMBINATION a–b	F_1 FEMALE	F_1 MALES			
		+	a	b	a.b
	d.n/c.l	12	4	0	2
		31	118	11	6
		31	18	290	87
		0	0	2	0
	d.c/cr	296	61	110	237
		279	51	128	235
	d.c/ho	17	16	27	98
		83	73	9	41
	d.c/ac	103	35	53	77
		105	12	65	74
	d.c/+	691	208	273	643
	d/c	172	303	505	151
d–l	*d/l*	107	220	413	135
d–wa	*d/wa*	207	195	126	152
d–el	*d/el*	432	482	214	216
d–sv	*d/sv*	133	78	142	81
d–ma	*d/ma*	69	75	62	75
d–Y	*d/Y*	100	107	116	111
d–sl	*d/sl*	230	265	306	185
d–ta	*d/ta*	151	139	166	115
d–n	*d.n/+*	333	111	50	124
	d/n	245	238	144	172
d–v	*d/v*	262	206	381	
d–st	*d/st*	188	140	183	125
d–wh	*d/wh*	294	222	242	228
d–cr	*d/cr*	406	298	407	286
d–in	*d/in*	93	97	70	101
d–td	*d/td*	67	78	60	69
d–ho	*d/ho*	44	114	92	114
d–ac	*d/ac*	156	112	170	86
w–r	*w/r*	261	168	260	204
w–f	*w/f*	616	550	375	366
w–c	*w/c.l*	69	98	21	10
		14	14	82	57
	w/c	139	161	149	116
w–l	*w/l*	127	161	138	105
w–el	*w/el*	201	151	81	201
w–sv	*w/sv*	157	76	155	47
w–Y	*w/Y*	155	92	95	85
w–ta	*w/ta*	180	171	185	143
w–n	*w/n*	54	40	27	27
w–ho	*w/ho*	170	150	169	133
r–m	*r/m*	416	615	242	

TABLE 2 (*Continued*)

COMBINATION a–b	F_1 FEMALE	F_2 MALES +	a	b	a.b
r–f	*r.f/+*	859	750	764	823
	r/f	743	930	699	472
r–c	*r/c.l*	736	611	92	82
		87	82	636	660
	r.c/n	18	9	103	49
		51	21	10	6
	r/c	1065	941	966	970
	r.c/+	69	30	113	55
r–l	*r/l*	1017	901	912	914
r–wa	*r/wa*	194	188	192	172
r–br	*r.br/sv*	76	81	48	45
		56	38	46	35
	r.br/+	282	295	206	163
	r/br	150	176	112	83
r–el	*r/el*	947	835	575	536
r–sv	*r/sv*	265	251	209	161
r–V	*r.V/+*	69	43	66	55
	r/V	93	75	79	71
r–sl	*r/sl*	313	322	290	318
r–ta	*r/ta*	149	133	119	97
r–n	*r/n*	749	713	405	285
r–sp	*r/sp*	259	291	200	178
r–v	*r/v*	140	92	51	
r–b	*r.b/+*	47	46	9	
	r/b	516	397	38	19
	r?			45	
r–st	*r/st*	49	57	33	35
r–wh	*r/wh*	109	94	122	114
r–ct	*r/ct*	233	222	183	179
r–gl	*r/gl*	111	695	763	108
r–in	*r/in*	141	157	111	91
r–td	*r/td*	93	152	145	
r–gy	*r/gy*	130	319	151	331
f–c	*f/c.l*	86	52	13	6
		8	5	106	41
	f/c	228	146	244	153
f–l	*f/l*	473	378	495	212
f–el	*f/el*	264	224	220	183
f–sv	*f/sv*	72	86	79	65
f–v	*f/v*	203	122	111	50
f–b	*f/b*	14	3	4	7
f–wh	*f/wh*	139	160	150	144

TABLE 2 (*Continued*)

COMBINATION a–b	F_1 FEMALE	F_2 MALES			
		+	a	b	a.b
f–ct	*f/ct*	112	57	116	33
f–gl	*f/gl*	140	139	196	72
f–in	*f/in*	294	135	142	103
f–ld	*f/ld*	86	74	73	4
f–cw	*f/cw*	130	136	131	128
c–l	*c.l/br*	184	19	12	182
		181	8	19	152
	c.l/Y	67	6	15	63
		42	3	3	23
	c.l/ta	141	7	19	138
		105	16	10	127
	c.l.n/+	742	160	1	14
		8	1	41	320
	c.l/+	5472	658	588	5131
	c/l	36	279	303	38
c–wa	*c/wa*	240	237	152	144
c–br	*c/br*	196	201	208	160
c–el	*c/el*	137	157	148	
c–sv	*c/sv*	148	128	174	141
c–ma	*c/ma*	200	399	173	
c–Y	*c/Y*	111	93	61	36
c–sl	*c/sl*	111	99	111	91
c–ta	*c/ta*	338	574	274	496
c–n	*c.n/+*	743	174	49	321
	c/n	92	531	227	35
c–v	*c/v*	157	185	145	128
c–st	*c/st*	128	141	115	112
c–wh	*c/wh*	337	359	615	
c–cr	*c/cr*	357	347	330	363
c–cl	*c/cl*	60	55	25	22
c–gl	*c/gl*	165	169	160	159
c–in	*c/in*	147	144	137	136
c–cw	*c/cw*	115	172	129	98
c–gy	*c/gy*	154	183	171	200
c–ho	*c/ho*	33	125	156	50
c–ac	*c/ac*	138	130	117	139
l–br	*l/br*	203	194	197	171
l–el	*l/el*	679	646	461	331
l–Y	*l/Y*	226	234	191	168
l–ta	*l/ta*	148	157	121	137
l–n	*l.n/+*	902	15	9	361
	l/n	19	449	182	2
l–b	*l/b*	117	116	11	0
wa–br	*wa.sl/br*	67	209	204	39
		86	173	220	47
	wa/br	269	725	744	167
wa–el	*wa/el*	169	162	110	127

TABLE 2 (*Continued*)

COMBINATION a–b	F_1 FEMALE	F_2 MALES			
		+	a	b	a.b
wa–sv	wa.sl/sv	83	107	113	80
		89	102	101	72
	wa/sv	289	341	338	260
wa–sl	wa.sl/+	467	435	496	394
wa–ta	wa/ta	83	98	77	41
wa–st	wa/st.wh	270	75	13	5
		24	6	261	79
	wa/st	294	81	274	84
wa–wh	wa/wh	645	352	628	343
wa–ct	wa/ct	148	99	90	43
wa–Eh	wa/eh	271	278	215	169
br–el	br.gy/el	31	34	585	311
		574	549	33	21
	br/el	787	797	828	403
br–sv	br/sv	296	194	205	175
br–sl	br/sl	276	243	259	267
br–ta	br/ta	161	124	147	150
br–b	br/b	196	170	47	17
br–st	br/st	193	167	197	206
br–gy	br.gy/+	616	345	607	570
el–sv	el/sv	163	145	181	99
el–Y	el/Y	412	337	235	84
el–sl	el/sl	305	144	159	120
el–ta	el/ta	545	481	473	425
el–n	el/n	145	99	71	86
el–sp	el/sp	199	60	178	22
el–tw	el/tw	423	296	263	152
el–cr	el/cr	216	181	216	
el–ct	el/ct	138	51	91	39
el–cw	el/cw	108	23	95	85
el–gy	el/gy	112	1157	1484	80
sv–ma	sv/ma	90	150	112	98
sv–sl	sv/sl	190	193	191	173
sv–ta	sv/ta	183	197	168	187
sv–n	sv/n	98	83	56	51
sv–v	sv/v	106	80	108	
sv–b	sv/b	246	194	60	74
sv–tw	sv/tw	95	138	64	62
sv–st	sv.st/+	95	61	92	101
	sv/st	260	167	160	156
sv–wh	sv.wh/cr	125	20	81	21
		93	40	71	15
	sv.wh/id	36	126	46	119
		136		121	27

TABLE 2 (*Continued*)

COMBINATION a–b	F_1 FEMALE	F_2 MALES +	a	b	a.b
	sv.wh/ek	116	34	87	52
		70	0	50	26
	sv.wh/+	567	343	467	350
	sv/wh	292	243	276	271
sv–cr	*sv/cr*	206	41	164	55
sv–cl	*sv/cl*	280	278	26	62
sv–gl	*sv/gl*	140	76	153	73
sv–td	*sv.(wh)/td.(wh)*	62	326	322	34
	sv.wh/td	36	126	136	
		46	119	121	27
sv–cw	*sv/cw*	438	125	354	125
sv–Eh	*sv/eh*	203	86	120	26
sv–ho	*sv/ho*	87	102	69	91
sv–ac	*sv/ac*	129	82	106	59
ma–sl	*ma.sl/+*	248	154	202	147
ma–ta	*ma/ta*	117	86	94	75
ma–wh	*ma/wh*	258	174	438	
ma–td	*ma/td*	85	194	178	54
Y–sl	*Y/sl*	185	108	138	117
Y–b	*Y/b*	14	17	1	0
Y–wh	*Y/wh*	138	118	149	120
Y–gl	*Y/gl*	46	53	73	67
Y–in	*Y/in*	162	141	114	63
sl–ta	*sl/ta*	467	436	377	399
sl–st	*sl/st.wh*	89	62	10	7
		13	7	60	72
	sl/st	102	69	70	79
sl–wh	*sl.wh/+*	264	252	231	239
	sl/wh	99	69	73	79
sl–gl	*sl/gl*	144	138	152	132
sl–in	*sl/in*	166	160	133	89
ta–sp	*ta/sp*	883	691	281	275
ta–b	*ta/b*	52	53	34	44
ta–tw	*ta/tw*	86	151	57	81
ta–wh	*ta/wh*	176	170	135	144
ta–ct	*ta/ct*	77	52	55	56
ta–gl	*ta/gl*	191	152	128	131
ta–cw	*ta/cw*	254	211	120	93
v–tw	*v/tw*	218	176	160	68
v–wh	*v/wh*	179	159	191	130
v–gy	*v/gy*	160	95	152	86
v–ho	*v/ho*	34	10	33	14
v–p	*v/p*	37	57	33	
st–wh	*st.wh/+*	496	45	50	472
	st/wh	60	406	400	55
st–sr	*st.sr/+*	172	162	169	200
st–ho	*st/ho*	98	91	89	71

TABLE 2 (*Continued*)

COMBINATION $a-b$	F_1 FEMALE	F_2 MALES			
		+	a	b	$a.b$
wh—cr	*wh/cr*	145	102	133	86
wh—ct	*wh/ct*	127	142	152	143
wh—gl	*wh/gl*	163	147	127	142
wh—td	*wh/td*	380	393	326	345
wh—gy	*wh/gy*	317	302	308	327
wh—Eh	*wh/eh*	150	139	70	76
cr—gy	*cr/gy*	85	85	75	76
cr—ho	*cr/ho*	92	99	80	116
ct—gl	*ct/gl*	117	127	139	117
ct—ho	*ct/ho*	131	123	111	119
gy—ho	*gy/ho*	126	127	120	113
gy—ac	*gy/ac*	36	215	174	31
Eh—ho	*eh/ho*	177	112	173	91

KEY TO TABLE 2

The list of genes given at the top of table 2 is arranged for convenient reference to the combinations presented in the left hand column. Combinations of kidney, *k*, made with each successive gene are presented followed by combinations of orange, *o*, made with each successive gene, et cetera. Thus if it is desired to know what tests if any have been made between any two genes, locate the group of combinations with the gene given first in the reference list. Combinations with succeeding genes will be found here in order.

The second column gives the mutant factors for which the F_1 females are heterozygous with / dividing contributions from the different parents.

The four succeeding columns (3 to 6) show the numbers of F_1 males arranged as wild-type, +(column 3), mutant type *a* (column 4), mutant type *b* (column 5), and the double mutant type *a.b* (column 6). In any given case factor *a* and factor *b* may be determined from column 1 ($a-b$). When a third mutant factor, *c*, appears in the formula of the F_1 female (column 2), males showing the trait are placed in their proper column (as regards *a* and *b*) in the next row below. When a fourth factor, *d*, appears in the F_1 formula, a third line is used, with a fourth line for the males showing both *c* and *d*. While *a* and *b* must be determined from column 1, *c* and *d* must be determined from the F_1 formula, in which *c* is placed so that it precedes *d*. Thus under combinations of *o* with *d* ($o-d$), we find F_1 female *o.d/sl.wh*. F_2 males are arranged as follows:

+	64,	*o*	63,	*d*	71,	*o.d*	66.
sl	75,	*o.sl*	92.	*d.sl*	48,	*o.d.sl*	37.
wh	103,			*d.wh*	128,		
sl.wh	139,			*d.sl.wh*	100,		

A vertical summary should give +, *o*, *d*, *o.d* (+, *a*, *b*, *a.b*) respectively, while a horizontal summary should give +, *sl*, *wh*, *sl.wh* (+, *c*, *d*, *c.d*) respectively, but in the present instance it is obvious that white-eyed wasps are indeterminable for orange. The white groups are therefore double and vacant spaces indicate masking effects. In the case of offspring from orange-ivory compound (o/o^i) females, orange males are recorded in + column.

Progenies of females heterozygous for more than two factors are in most cases included also under each possible combination of two. Thus the 636 males from $o^i.d/st$ are included with the 2528 from $o^i.d/+$, the 706 from o^i/st, and are repeated for d/st.

BIOLOGICAL AND STATISTICAL SIGNIFICANCE OF DATA

Let *AB*, *aB*, *Ab*, and *ab* represent the frequencies of the four phenotypes of offspring expected from heterozygous females *a.b*/+ (coupling test) or *a*/*b* (repulsion test). It may be supposed that wild-type, *AB*, is the most viable, but somatic overlapping may increase the relative number of *AB* and of *aB* phenotypes at the expense of *Ab* and *ab* respectively, or the reverse may occur. It may also happen that *AB* will be increased at the expense of *aB*, while *Ab* is not increased at the expense of *ab*. This may be due to the influence of factor *B versus b* or to a modifier of trait *A versus a* linked with factor *B* or *b*. A factor preventing overlapping acts as a "differentiator" (BRIDGES 1919, p. 268).

AB may excede *aB* due to differential viability in which case we might expect a comparable excess of *Ab* over *ab*. However, the ratio ab/AB cannot be predicted from $\frac{aB}{AB} \times \frac{Ab}{AB}$ since in some cases the double mutant type fails to be reduced proportionally to the single while in others the double may be highly lethal although one or both of the singles may show viability equal to that of wild-type under the conditions of culturing.

Linkage of one of the genes (*A* or *a* for example) with a lethal or semilethal may cause an excess or a deficiency of a mutant type of normal viability. Thus *aB* may surpass *AB* or the reverse. In this case we may expect a corresponding shift between *ab* and *Ab*, but such may not be obvious because of viability differences or somatic overlaps between the various combinations of *A* and *a* with *B* and *b*, or because of linkage of a second lethal or semilethal with *B* or *b*. While lethals are invisible in the material of table 2, semilethals may be either visible or invisible. Complete lethals, dying as pupae, which may be identified as to a second trait (eye color for example), have been shown in Habrobracon.

In case of linkage between *a* and *b*, $\frac{AB}{AB+aB}$ might be expected to

equal $\frac{ab}{Ab+ab}$ were it not for one or more of the three disturbing factors,—somatic overlapping, non-proportionate differential viability, and linked lethals or semilethals.

It is obvious that what has been said as to the relations of *a* and *b* may also apply to relations of either *a* or *b* with a third factor *c*, or a fourth factor, *d*. It is sometimes necessary to make comparisons of pairs of frequencies as regards *a* and *b*, separately for groups *CD*, *cD*, or *Cd* since either *c* or *d* may affect phenotypic ratios of *a* or *b*.

Table 1 has been prepared in order that the reader may readily estimate the statistical significance of various pairs of frequencies occurring in the summaries of table 2. By dividing the lower frequency by the sum (n) a percentage is obtained deviating more or less below 50 percent. (P = .90) are those percentages at or below which 10 percent of the samples of a given size (n) will be expected to fall, while (P = .99) are those percentages at or below which 1 percent of the samples of corresponding size will fall. The reader may then be 90 percent sure that there is a significant difference from equality between the two frequencies if the percentage is as low as or lower than (P = .90) and he may be 99 percent sure if it is as low as or lower than (P = .99). Whether these statistically significant deviations indicate somatic overlapping, differential viability, linked lethals, linkage of the two genes under consideration, or some combination of these conditions must be judged in the individual case from the nature and variability of the traits and comparison of the different percentages with each other and with the relation of the factors in the F_1 parent.

Whether there is linkage and what may be the best estimate of the percentage of crossovers cannot necessarily be determined from the ratio $\frac{AB+ab}{AB+aB+Ab+ab}$.

MULLER (1916) has shown convenient methods of determining gametic ratio, recombinations/straights, which compensate for viability differences. If viability be affected proportionally by two pairs of allelomorphs regardless of their combination and if the numbers of the genotypes be *AB*, *aB*, *Ab*, and *ab* respectively, the gametic ratio, r/s, equals $\sqrt{\frac{AB \times ab}{aB \times Ab}}$ for the repulsion test and $\sqrt{\frac{aB \times Ab}{AB \times ab}}$ for the coupling test. If, however, the factors differ in their effects on viability according to their various combinations with each other and if genotypes from the repulsion cross be AB_1, aB_1, Ab_1, ab_1 and from the coupling cross AB_2, aB_2, Ab_2, ab_2, then gametic ratio should be equal to any one of the four expressions:

$$\sqrt{\frac{AB_1 \times Ab_2}{AB_2 \times Ab_1}},\ \sqrt{\frac{AB_1 \times aB_2}{AB_2 \times aB_1}},\ \sqrt{\frac{ab_1 \times Ab_2}{ab_2 \times Ab_1}},\ \sqrt{\frac{ab_1 \times aB_2}{ab_2 \times aB_1}}.$$

This second method, the method of reverse crosses, does not compensate for linked lethals, but comparison may be made of the six ratios obtainable (two by first method in case reverse crosses are made and four by second) and in case of differing results presence of linked lethals determined.

MULLER's method does not compensate for somatic overlaps or for difficulty in determination of types, in which case phenotypic ratio involves not only deficiency of certain types below gametic ratio but also excess of other types. If viability difference be absent between A and a and if overlapping be from a to A in the same proportion whether B or b be present, then the phenotypic distribution AB, aB, Ab, ab may be corrected as follows. Decrease AB by subtracting $\frac{AB+Ab-aB-ab}{2(AB+Ab+aB+ab)} \times aB$ and increase aB by the same amount. Also decrease Ab by subtracting $\frac{AB+Ab-aB-ab}{2(AB+Ab+aB+ab)} \times ab$ and increase ab by the same amount. In other words each of the two numerically deficient groups should be increased at the expense of its corresponding excess group by a proportion of itself equal to one-half the difference between the sum of the deficient groups and the sum of the excess groups to the total.

The gametic ratio calculated after this correction is made will in case of linkage always give fewer recombination types than the original data. If overlapping of one difference be always in the same direction and unaffected by the other difference or correlated differences, the gametic ratio obtained should be correct. Even if overlapping be in both directions (from A to a as well as from a to A), recombinations after correction should be fewer than before. True crossover value will not be greater than that indicated by ratio thus obtained, but it may be considerably less. The value of the method lies in the fact that it gives a maximum which is lower than may be calculated from the frequencies directly.

DISCUSSION OF DATA

DUNNING (1931) reports a female-sterile mutant type with bar eyes. Her data indicate independent segregation with orange, defective, reduced and eyeless. With reference to the last, F_2 from eyeless females by bar males consisted of wild-type 132, bar 86, eyeless 70. Bar and eyeless were both of decreased viability as compared with wild-type and it is possible that the double mutant type, bar eyeless, were lethal, as suggested by Dr. DUNNING, or that some of the F_2 eyeless phenotypes were genetically eyeless bar.

Comments will be made on data of table 2 in those cases only which involve marked deviations from expectation or other points of interest.

Kidney shows unmistakable linkage with orange. Combinations of the various allelomorphs may be summarized without regard to other genes. From $k^s.o^i/o$ females, progeny were orange 292, small orange 253, ivory 207, small ivory 327; 41.48 percent recombinations among the full-sized and 43.62 percent among the small. From $k^s.o^i/+$ (omitting those from $k^s.o^i/m$) progeny were wild-type 847, small 757, ivory 436, small ivory 1127; 33.98 percent recombinations among the full and 40.18 percent among the small. Small overlap with full and modifying factors influence counts, as may be noted among offspring from $k^s.o^i/sl$ among which semilong had unusually high proportion of small eyes. From $k.o^i/+$ females, progeny were wild-type 489, kidney 198, ivory 314, kidney ivory 359; 39.10 percent recombinations among the full and 35.55 percent among the kidney. Kidney is semilethal when wasps are reared at 30°C and hence counts show deficiency. Eyes are frequently lacking and identification by ocelli alone may account for disparity in ratio of recombinations. From $k^s.o^i/o$, progeny were orange 67, extreme-small orange 11, ivory 45, extreme-small ivory 55; 41.17 percent recombinations among the full and 16.67 percent among the extreme small. It is impossible to tell with certainty the difference between orange and ivory among the extreme-small. From $k^c.o^i/+$, progeny were wild-type 623, extreme-small 294, ivory 433, extreme-small ivory 672; 41.00 percent recombinations among the full and 30.43 percent among the extreme-small. The difference between black and ivory can be determined among extreme-small, but when eyes are lacking identification by ocelli is unreliable. The most reliable data for determining percentage of crossing over are therefore the counts of full-sized among fraternities segregating kidney or extreme-small. These total 1971 with 792 (40.18 percent) crossovers.

Summarized combinations of orange and miniature ($o-m$) show 499 (11.05 percent) crossovers among the 4515 non-miniature; 265 (11.00 percent) among the 2399 adult miniature; and 51 (13.42 percent) among the 380 lethal pupae. Crossing over may therefore be taken as approximately 11 percent. Ratio of pupae to adults is of no significance as pupae were not always counted. Ratio of adult miniature, 2399, to non-miniature, 4515 (53.13 percent), expresses relative viability of miniature.

A three point experiment planned to determine relative positions of kidney, orange and miniature yielded 1803 males from $k^s.o^i/m$ females. There were 55 percent with small eye, 56 percent ivory, and 43 percent miniature. Difficulty in identification of small makes counts unreliable. Miniature, more numerous on the average than expected, were not equally deficient in the various alternative classes. Summary of alternative classes

shows straights 1119, recombinations between small and ivory 632, between small and miniature 548, and between ivory and miniature 188. From this it may be judged that miniature lies between orange and kidney, giving 136 (7.54 percent) crossovers between orange and miniature alone, 496 (27.51 percent) between miniature and kidney alone, and 52 (2.88 percent) double crossovers. From these data it may be concluded only that miniature lies at about one-fourth the distance from orange to kidney.

No linkage is indicated in combinations between kidney and any genes tested other than orange and miniature. Linkage of cantaloup and long and thermo-lethality of kidney may be noted among progeny from *k.r./c.l.* Small eye, k^s, depart widely from expectation due to overlapping and as affected by modifiers. Maroon is masked by ivory (from $k^s.o^i/ma$). Small is masked by eyeless (from k^s/el).

Mutant type offspring from k^s/el were classified into three groups,—extreme-small 503, eyeless with lobes on sides of head 659, and eyeless without the lobes 289. Significance of these types is uncertain and the problem is being further investigated.

Proportion of types from k^s/v is best explained by overlapping of small and decreased viability of vestigial.

Offspring of k^s/cr demonstrate that crescent is not allelomorphic with kidney. The k^s/cr females had eyes of approximately normal size.

Under combinations of orange with defective venation ($o-d$), it may be seen that defective is masked by reduced (from $o.d.r/+$); maroon is masked by ivory (from $o^i.d/ma$); orange is masked by white (from *o.d/sl.wh*); defective is masked by vestigial (from *o.d/v*).

Defective are of normal viability but overlap with wild-type and the trait may appear as a result of minor factors without the presence of *d*. This factor was called d_{1195} in a study of variation and modifiers (WHITING 1924).

Among progeny from *o.d/sl.wh* there appears to be linkage between *d* and *sl*, but this is a somatic effect. (See summary under $d-sl$.) Semilong contracts the end of the wing (so that vein R_4 is more likely to extend from R_3 to M_1) thus obliterating the effect of *d*. The excess of defective as compared with type is undoubtedly an effect of minor factors. Eyeless has an effect on defective somewhat similar to *sl* (from *o.d/el*) but not so extreme. (See $d-el$.)

Summaries of combinations of orange and defective ($o-d$), including much material previously published, indicate no linkage but show overlapping of defective into wild-type.

Wrinkled are somewhat less viable than wild-type, frequently failing to eclose. There were 4540 (48.6 percent) recombinations with orange among 9339.

Progeny from $o^i.r/m$ show linkage of ivory and miniature and low viability of miniature. Reduced were not classified as regards miniature. Lethal miniature pupae can be identified as to eye color and show linkage with orange. The lethal pupae include reduced miniature as well as miniature which accounts for the deficiency of adult reduced as compared with ivory reduced.

Among progeny from $o^i.r/o.b$, beaded were not classified as regards reduced. Beaded are of very low viability.

Summary of $o-r$ shows 6498 straight and 6477 recombination.

Fraternities bred from $o.m/c.l$ (by C. H. BOSTIAN) show linkage of o and m (14.3 percent crossovers, vertical summary) and of c and l (7.1 percent crossovers, horizontal summary). Summarizing for miniature and long alone we have wild-type 768, miniature 405, long 936, miniature long 236, suggesting linkage (41 percent recombinations). Among the 1704 non-miniature there are 45.07 percent recombinations while among the 641 miniature there are 36.82 percent recombinations. Both of these ratios differ significantly from 50 percent (table 1), and are also widely different from each other. The case can be explained by linkage of a lethal with non-long, and by increased lethality of miniature by long. Other evidence shows that there is no linkage between miniature and long.

Among combinations $o-c$ from $o.c/el$, eyeless were classified by ocelli as black or light, the latter including orange, cantaloup, and orange cantaloup.

Among combinations $o-el$, progeny from $o.el/sp$ show majority of eyeless classified as non-spread. Spread wing lightens muscle-insertion pigment on side of thorax and wasps are classified by this trait. Eyeless has a general darkening effect on body pigment which counteracts this character.

Progeny of $o.cw/el$ show majority of eyeless classified as crepe-wings. Eyeless causes a certain amount of wrinkling of wings resulting in confusion.

Summaries from orange (or ivory) crossed with eyeless show F_2 males,—wild-type 1136, orange (or ivory) 1071, eyeless with black (wild-type) ocelli 343, eyeless with light ocelli 346, eyeless with ocelli unclassified 441, giving no evidence of linkage. Eyeless are of decreased viability as compared with non-eyeless sibs.

Progeny of $o.cw/sv$ show deficiency of shot-veins but among crepe-wings there is apparent equality. This is because extreme shot-veins may cause sufficient wing deformity to be classified as crepe-wings. Shot-veins is a variable trait, much affected by external conditions, and overlapping with wild-type.

Maroon eye (masked by ivory or by white and classified with uncer-

tainty in presence of orange) may segregate in equal numbers from type (from o^i/ma) or may surpass (from $o^i.k^s/ma$) or fall below (from o/ma, $ma.sl/+$, ma/wh, ma/ta) type. These deviations may be due to linked lethals. Maroon is linked with shot-veins and with truncated.

Under $o-n$, progeny from o^i/n show a significant deficiency of ivory among the narrow despite a slight excess of ivory among the non-narrow. Progeny from o/n indicate no linkage and there is no reason to suppose that ivory would itself reduce viability of narrow. Possibly there was coupled with ivory, a factor having such an effect.

Under $o-Eh$, progeny from o^i/Eh show a significant excess of ivory, similar both in extended head and in non-extended head. This is probably due to a linked lethal.

As regards $d-r$, reduced masks defective. The 626 reduced from d/r include 42 with R_4 complete in both wings and 584 with R_4 lacking from one or both wings. Lack of R_4 in reduced is no criterion of presence of factor d.

Various combinations of d, c, l, n, cw, and ho show linkage. On account of overlapping and modifiers defective show much irregularity. Low viability of narrow also disturbs ratios.

Progeny of $d.c/ho$ show that honey tends decidedly to increase proportion of defective. This is due to general lightening of wing veins along with body pigment. If this group be omitted there are 1451 progeny from $d.c/+$,—wild-type 591, defective 119, cantaloup 237, and defective cantaloup 504. Cantaloup are in slight excess while defective are 42.7 percent. Apparent crossovers are increased among cantaloup, decreased among non-cantaloup by overlapping of defective into wild-type. Correction according to the method above suggested gives probable genotypes as follows: +571.4, d 138.6, c 154.1, $d.c.$ 586.9. Crossover ratio by square root of product method is from uncorrected data 23.6 percent, from corrected 20.1 percent.

Defective is linked with long. From d/l the 875 progeny show deficiency of cantaloup (40.6 percent) and excess of long (62.6 percent) on account of overlaps and linkage with narrow. Apparent crossovers are 32.7 percent among non-long and 25.0 percent among long. The true value cannot be determined.

From $d.n/+$ the 618 progeny include 38.0 percent defective and 28.2 percent narrow. Apparent crossovers are 25.0 percent among non-narrow and 28.7 percent among narrow. From d/n the 799 progeny include 39.5 percent narrow and 51.3 percent defective. Evidently minor factors for defective are present which explain the equality of defective phenotypes. One of these may be linked with narrow since narrow defective exceeds narrow.

From $c.l/+$ the 11849 progeny include 48.9 percent cantaloup and 48.3

percent long. Crossovers are 10.5 percent. From *c/l*, the 656 progeny include 48.3 percent cantaloup and 52.0 percent long. Crossovers are 11.3 percent.

From *c.n/+* the 1287 progeny include 38.5 percent cantaloup and 28.7 percent narrow, the deficiency of both being due to low viability of narrow. Crossovers are 16.0 percent. From *c/n* the 885 progeny include 64.0 percent cantaloup and 29.6 percent narrow, the excess of the former being due to repulsion from narrow. Crossovers are 14.0 percent.

From *c/cw* the 514 progeny include 52.5 percent cantaloup and 44.2 percent crepe-wings. Crossing over is 41.6 percent.

From *l.n/+* the 1287 progeny include 29.2 percent long and 28.7 percent narrow, the deficiency of both being due to coupling and reduced viability of narrow. From *l/n* the 651 progeny include 69.3 percent long and 28.1 percent narrow, the excess of the former being due to repulsion from narrow. Crossovers are 2.0 percent in the coupling cross and 2.1 per cent in the repulsion cross.

The 1287 progeny from *c.l.n/+* indicate that the order of genes is *c−l−n* with 203 (16.6 percent) crossovers between cantaloup and long, 24 (1.86 percent) between long and narrow. There are 2 (0.16 percent) doubles with 0.30 percent expected. Alternative classes show deficiency of narrow in each case.

The 612 progeny from *d.n/c.l* indicate the order as *c−l−n−d*. There are 68 (11.11 percent) crossovers between *c* and *l*, 20 (3.27 percent) between *l* and *n*, 155 (25.33 percent) between *n* and *d*. Doubles are 2 (0.33 percent) for *c−l* and *l−n* with 0.36 percent expected; 31 (5.07 percent) for *c−l* and *n−d* with 2.81 percent expected; 8 (1.31 percent) for *l−n* and *n−d* with 0.83 percent expected; and triples are 2 (0.33 percent) with 0.09 percent expected. The differences between actuality and expectation for *c−l* and *n−d* and for *l−n* and *n−d* are statistically significant suggesting negative interference. However, narrow are in each pair of alternatives numerically deficient and defective overlaps with non-defective. Hence proportion of apparent alternative pairs to each other is modified.

From *d.c/ho*, the 364 progeny include 62.6 percent defective, 48.1 percent cantaloup and 56.6 percent honey. Crossing over is 22.5 percent between honey and cantaloup. Since honey causes a large proportion to be counted as defective, defective cantaloup honey phenotypes are greatly increased at expense of cantaloup honey, and defective honey phenotypes at expense of honey. The 158 non-honey include 44 (27.8 percent) recombinations between honey and defective, 43 (27.2 percent) between cantaloup and defective, and 33 (20.8 percent) between honey and cantaloup, giving no basis for relative positions of the three genes.

Progeny of *w/el* show apparent increase of wrinkled eyeless at expense of eyeless because eyeless tends to wrinkle wings.

Combination *r*—*f* shows linkage. The 3196 progeny from *r.f/+* show 46.5 percent crossovers among non-fused and 48.0 percent among fused. The barely significant difference is probably due to low viability of reduced fused. Reduced are 49.2 percent of total and fused are 49.6 percent of total. The 2844 progeny from *r/f* show 44.4 percent crossovers among non-fused and 40.3 percent among fused. The difference is apparently due to low viability of reduced fused. Reduced are 49.3 percent of total while fused are only 41 percent of total. On account of lowered viability of fused in the repulsion cross, perhaps due to a linked lethal, it is preferable to derive crossover ratio from non-fused. Among 3282 non-fused from reverse crosses there are 45.5 percent crossovers.

Combination *r*—*gl* shows linkage. The 1677 progeny from *r/gl* show 13.1 percent crossovers.

Combination *f*—*gl* shows linkage. Among the 547 progeny from *f/gl*, fused were deficient (38.6 percent) while glass were normally viable (49 percent). Wild-type and fused were equal illustrating reduced viability balanced by linkage. Fused glass were very deficient as here linkage and lowered viability act in the same direction. Among the non-fused, crossovers are 41.7 percent while among the fused, crossovers are 34.1 percent. Crossovers by square root of product method are 37.7 percent.

Combination *r*—*b* shows great lethality of beaded.

Reduced were significantly low among progeny from *r/b* and also from *r.c/n*, *r/el* and from *r/v* (in which case vestigial masks reduced). From *r/m*, miniature were not separated among the reduced although this might have been done since reduced does not really mask miniature. Among progeny from *r/td*, reduced were in significant excess, but *td* masks reduced. From *r/gy*, reduced were over twice as frequent as non-reduced. These significant deviations of reduced in certain crosses indicate linked lethals.

F_2 males from fused usually show deficiency of fused, but those cases in which fused approximately equal non-fused indicate that dead have been removed from cocoons and counted. The group from *f/td* show reduced viability of the double recessive.

Eyeless masks cantaloup (from *c/el*) and cantaloup masks maroon (from *c/ma*).

Progeny from *c/ta* show a striking excess of cantaloup indicating one or more linked lethals. From *c/gy* there was a slight excess of cantaloup which may be similarly interpreted.

Wavy and broad show linkage. Among 1905 males from *wa/br* there are 26.5 percent crossovers among non-wavy and 18.7 percent among wavy. By square root of product method crossovers are 22.4 percent.

Progeny of *wa/sv* suggest loose linkage (44.7 percent). Progeny of *br/el* (including *br.gy/el*) show low proportion of broad eyeless. The excess of eyeless non-broad over broad non-eyeless suggests that broad overlap with non-broad among the eyeless, but reduced viability or linkage may also in part explain the proportion. Gynoid is linked with eyeless (from *br.gy/el* included also under *el − gy*). Crossovers are 6.6 percent. The low number of broad non-gynoid from *br.gy/+* is thus explained.

Among progeny from *el/sp*, eyeless were unusually low. Spread cannot readily be distinguished among the eyeless which have a general darkening of body pigment obscuring the light pleural mark characteristic of spread.

Only three classes appear from *el/cr*, the double mutant type, eyeless crescent, being either masked or lethal.

The low proportion of eyeless non-crepe-wings from *el/cw* is because eyeless wrinkles wings somewhat, causing confusion. Many of those classed as eyeless crepe-wings should therefore be classed as eyeless.

Shot-veins is linked with maroon. The 450 progeny from *sv/ma* show 42.0 percent crossovers, 37.2 percent among non-maroon and 46.5 percent among maroon.

Shot-veins is variable with much overlapping and modification by environmental and genetic factors. It has the curious effect in combination with white of producing red spots in the white eyes especially in the ventral regions. Thus in fraternities here reported the double recessive, shot-veins white, is variegated. Variegation is of value in determining the presence of the factor for shot-veins even though the trait is masked in the wings, as by truncated which proves to be linked with shot-veins. Thus among progeny from *sv.wh/td* which is included both under *sv − wh* and *sv − td*, the black-eyed wasps could be separated for shot-veins only if they are non-truncated while white shot-veins truncated could be separated by eyes from white truncated. This fact was utilized in an experiment to test linkage of shot-veins and truncated in which white was present in both parental types. It may be noted that in combinations *sv − td* crossovers are less frequent in truncated than in non-truncated, perhaps due to decreased viability of the double recessive or to failure in counting to recognize variegated in all cases. From *sv. (wh)/td. (wh)* crossovers are 12.4 percent; —16 percent among non-truncated and 9.5 percent among truncated. From *sv.wh/td* crossovers are 22.2 percent among non-white non-truncated, 27.8 percent among white non-truncated and 18.2 percent among white truncated. The white in this cross average 22.7 percent crossovers.

Maroon and truncated are linked. From *ma/td* there are 26.8 percent crossovers. Shot-veins, truncated and maroon thus form a linkage group with genes arranged in that order.

Variation in ratio of shot-veins may be noted in combinations with other

traits. With semilong, tapering, narrow, and possibly honey, shot-veins are approximately equal to non-shot-veins whether or not in combination with the other mutant factor. With beaded and stumpy, shot-veins deviate significantly below non-shot-veins except when in combination with the other mutant factor which may act as a differentiator. With twisted, however, there is a significant deviation in the reverse direction. Vestigial masks shot-veins. Significant deviations of shot-veins below non-shot-veins occur in combination with white, crescent, glass, crepe-wings, extended-head, and aciform. Club increases number counted as shot-veins because club itself affects venation in a somewhat similar although more restricted way and hence some club were classified as club-shot-veins.

Progeny from *sv.wh/Eh* are in unexpected proportions which apparently cannot be explained without elaborate hypotheses. No linkage of the genes under consideration is indicated. Total absence of shot-veins extended-head is far from expectation on the basis of ratio of single mutant types to wild-type.

Tapering significantly exceeds non-tapering from *ta/tw*. The difference probably due to a linked lethal, is less among twisted than among non-twisted, probably because of reduced viability of the double recessive.

Stumpy and white are linked showing 9.0 percent crossovers in the coupling cross and 12.4 percent in the repulsion.

There is an indication of possible though very loose linkage between stumpy and strap (from *st.sr/+*).

Gynoid and aciform are linked with 14.7 percent crossovers (from *gy/ac*).

AZYGOSIS IN OTHER FORMS

Segregation of gametic characters such as chromosome differences, has been shown by direct observation to result in numbers approximating the 1:1 ratio. Besides the extensive records on distribution of sex chromosomes in meiosis segregation of heteromorphic homologous chromosomes in Acridians has been reported by CAROTHERS (1926). It is well known that spermatids of many forms may be counted directly for presence or absence of the X chromosome. Measurements of sperm heads have been shown (ZELENY and FAUST 1915) to form a bimodal frequency distribution indicating presence or absence of the X.

Microspore characters in plants are for the most part parental traits having much the same relation to the nuclei within that the pericarp of grain, the color of an egg shell or the direction of coiling of the shell of Lymnaea (STURTEVANT 1923) have to their respective embryos. A few pollen characters, however, have been observed to segregate. (See SANSOME and PHILP 1932, for discussion and bibliography.) Bimodal curves of size of pollen grain have been correlated with sex chromosomes in Cannabis,

Melandrium and Rumex. A great variety of pollen lethals, due to chromosome irregularities, may be directly identified by form and size of the grains, as in Datura, maize and Oenothera. Type of carbohydrate storage has also been noted in pollen of rice and maize, which by color reaction after iodine test may be separated into two distinct types in 1:1 ratio. Dimorphism of pollen determined by segregating genetic factors has been noted also in Oenothera in respect to form of starch grains.

Difference in rate of growth of pollen tubes of several plants (Oenothera, Melandrium, Rumex, Triticum, Datura, Zea) according to segregating genetic factors may be directly observed. This "certation" explains many cases of self- and cross-incompatibility.

In fungi and mosses as well as to some extent in algae, it is often possible to separate the spores of a tetrad derived from a single spore mother cell and to study the traits of the azygotes developed. This method of tetrad analysis is invaluable in the study of synapsis phenomena (GOLDSCHMIDT 1932).

ALLEN (1930) has shown segregation of traits in dioecious leafy liverworts. Bryophyte plants are azygous and haploid.

Sporophytes derived from self-fertilization of a monoecious moss plant or of a fern prothallium should be comparable with diploid azygotes in being completely homozygous. ANDERSSON-KOTTÖ (1927), working with frond characters of *Polystichum angulare*, cultivated the prothallia from each spore in isolation and found that all the sporophytes from one prothallium were alike and homozygous. Thus segregation of prothallia for two genic differences affecting fronds was noted.

Haploid sporophytes of seed plants are occasionally found resulting from development of an unfertilized egg nucleus. These azygotes are usually small, weak, and highly infertile. Self-fertilization results in completely homozygous offspring, but such are more readily obtained asexually following injury to growing points (LINDSTROM 1929). There is thus no real genetic difference to be expected between the former (zygotes) with chromosome number regulated by fertilization and the latter (azygotes) with chromosome number regulated asexually.

Haploid parthenogenesis probably occurs in frogs (PARMENTER 1926) as a result of artificial stimulation. Both haploid and diploid mitotic figures were found in impaternate tadpoles and in cleavage nuclei, in some cases in the same individual, indicating "that the diploid number is not always formed before the first cleavage." All frogs that metamorphosed (25) were diploid. Reconstitution of diploid number is indicated but "it is still possible that the diploidness of these viable individuals may have been produced before the first cleavage." If numerical regulation occurred between

the second maturation division and the first cleavage (as apparently in the honey-bee) these impaternate diploid frogs would still be azygotes.

Haploid parthenogenesis occurs in a number of invertebrates. The resulting azygotes are male in Hymenoptera, Aleurodidae, Coccidae, thrips, mites, and rotifers.

Azygotic ratios have been shown in drone honey bees. NEWELL (1914) made reciprocal crosses of yellow Italian with gray Carniolan. The heterozygous yellow queens produced yellow and gray drones segregating in equal numbers. MICHAILOFF (1931) reported a recessive mutation to white eyes. Heterozygous black-eyed queens produced black-eyed and white-eyed drones in equal numbers. Males of several Hymenopterous insects are haploid at least with respect to the spermatogonia, but a diversity of opinion prevails with regard to the honey-bee. NACHTSHEIM (1913) regards the males as essentially haploid, but although the mature egg nucleus contains but eight chromosomes these become sixteen on the cleavage spindle. The embryo has 16, 32, or 64 in various tissues. Spermatogonia contain 16 chromosomes. First spermatocytes have 16 dyads, which fail to divide in the first maturation division, but fuse in pairs to form bivalents. These divide in the second maturation division so that the spermatids have 8 dyads or 16 chromatids. The male pronucleus therefore has 16 chromosomes. Since the 8 chromosomes of the mature egg nucleus divide into 16, the fusion nucleus has 32. NACHTSHEIM regards the eight chromosomes of the maturation divisions as compound, "Sammelchromosomen," and believes that 16 is the fundamental number (potential linkage groups). Since however there are eight tetrads in the first ovocyte and eight bivalent dyads (tetrads) in the second spermatocyte, the number eight may be regarded as haploid (potential linkage groups) while the 16 of the ovogonia or of the spermatogonia represent eight homologous pairs (diploid). Somatic cells of males or of females may contain two, four, or eight sets of homologues rather than fragments of the original eight chromosomes.

This process of doubling of chromosome number may be regarded as one method of regulation in order that chromatin (and genic) content may be increased. Such multiple sets have been shown by HOLT (1917) in the large larval digestive cells of Culex in which cytokinesis is inhibited during the pupal stage. Another and more usual method of regulation, probably not essentially different physiologically from numerical increase, is to enlarge size of chromosomes either in haploid or in diploid number according to the various cell sizes. Thus the haploid number is retained in many azygotes. This process of regulation is very poorly carried out in haploid sporophytes which are consequently weak and of small size.

Azygotic females probably occur in a number of invertebrates, but fe-

males of most thelytokous races and species produce their impaternate daughters by some form of diploid parthenogenesis either by suppression of second ovocyte division with or without preceding asynapsis or by refusion of second polar body with egg nucleus.

Facultative thelytoky occurs in several orthopterous insects. In the grouse locusts, Tettigidae, both genetic and cytological studies indicate that this is haploid. Virgin females heterozygous for various genes governing color patterns of pronotum and hind femora produce daughters which are in all cases (with one or two doubtful exceptions) homozygous. Cytological studies of embryonic cells show numbers varying from seven (haploid) to fourteen (diploid) chromosomes. Chromosomes of the haploid set are in transverse thickness twice the size of the diploid, so that each additional above seven in number means that one thick has been replaced by two thin. These facts seem to indicate regulation of chromatin volume followed by reconstitution of diploid number by longitudinal division. ROBERTSON (1930) and NABOURS (1930) hold that the diploidal condition is retained or restored by suppression of the second ovocyte division and that the thick chromosomes are dyads persisting into embryonic cells after diploid parthenogenesis. KING and SLIFER (1933), however, state that the diploid condition is restored in other grasshoppers after haploid parthenogenesis and that in grouse locusts, if diploid parthenogenesis resulting from suppression of second maturation division occurs, the first maturation division must be completely segregational. Since this is highly improbable and since it is unlikely that dyads should persist as such into late embryonic stages, it is better to regard these impaternate females as diploid azygotes.

SUMMARY

Azygotes are defined as impaternate organisms developed from cells (eggs or spores) which have undergone complete reduction in meiosis (haploid parthenogenesis). Azygotes may be haploid, or through chromosome splitting, diploid, tetraploid, et cetera.

The ratio in which azygotes from a heterozygous parent appear may depart widely from the gametic ratio because of masking effects, overlapping, and viability differences, but especially because of linked lethal factors to which zygotes from backcross data are relatively immune. A method for partially correcting somatic overlapping is given.

According to the work of other investigators, gametic segregation may be directly observed in chromosome differences in gametogenesis and in measurements of sperm-head size. Azygotic segregation may be noted in certain pollen-grain characters, pollen-tube growth, gametophytes from single spores in fungi, mosses and ferns, drones of honey-bees and impaternate

females of grouse locusts. Haploid parthenogenesis in seed plants and frogs is cited with numerical doubling in some cases.

The honey bee is regarded as an extreme example of this numerical increase since the haploid number, eight, occurring only in the gametocytes, is doubled in male and female pronuclei and further increased in somatic mitoses.

Azygotic ratios in Habrobracon from counts of about 186,000 males from mothers heterozygous for two or more genes are given. These involve 254 different combinations of 39 genes.

Seven linkage groups are shown with genes as follows:

1. Orange, miniature, kidney. 2. crepe-wings, honey, cantaloup, long, narrow, defective. 3. reduced, glass, fused. 4. wavy, broad. 5. eyeless, gynoid, aciform. 6. shot-veins, truncated, maroon. 7. stumpy, white. Data are not as yet sufficient to determine the exact relationships within these groups.

LITERATURE CITED

ALLEN, C. E., 1930 Inheritance in a hepatic. Science **71**: 197–204.

ANDERSSON-KOTTÖ, I., 1927 Notes on some characters in ferns subject to Mendelian inheritance. Hereditas **9**: 157–168.

BRIDGES, CALVIN B., 1919 The genetics of purple eye color in Drosophila. J. Exp. Zool. **28**: 265–305.

CAROTHERS, E. ELEANOR, 1926 The maturation divisions in relation to the segregation of homologous chromosomes. Quart. Rev. Biol. **1**: 419–435.

DUNNING, W. F., 1931 A study of the effects of X-ray radiation on occurrence of abnormal individuals, mutation rate, viability and fertility of the parasitic wasp, *Habrobracon juglandis* (Ashmead). Genetics **16**: 505–531.

GOLDSCHMIDT, RICHARD, 1932 Prä- oder Postreduktion der Chromosomen? Die Naturwissenschaften **20**: 358–362.

HOLT, CAROLINE M., 1917 Multiple complexes in the alimentary tract of *Culex pipiens*. J. Morph. **29**: 607–627.

KING, ROBERT L. and SLIFER, ELEANOR H., 1933 Genetic analysis of parthenogenesis in Nabours' grouse locusts. Amer. Nat. **67**: 80–81.

LINDSTROM, E. W., 1929 Variation in pure-line diploids derived sexually and asexually from haploid tomatoes. Anat. Rec. **44**: 286.

MIKHAILOFF, A. S., 1931 Über die Vererbung der Weisäugigkeit bei der Honigbiene (*Apis mellifera*). Z.I.A.V. **59**: 190–191.

MULLER, H. J., 1916 The mechanism of crossing over. Amer. Nat. **50**: 352–354.

NABOURS, ROBERT K., 1930 Induced parthenogenesis and homozygosis. Science **72**: 502–503.

NACHTSHEIM, HANS, 1913 Cytologische Studien über die Geschlechtsbestimmung bei der Honigbiene (*Apis mellifica L.*). Arch. f. Zellf. **11**: 170–241.

NEWELL, W., 1915 Inheritance in the honey-bee. Science N. S. **41**: 218–219.

PARMENTER, C. L., 1926 The chromosomes of parthenogenetically developed young tadpoles and early cleavages of *Rana pipiens*. Anat. Rec. **34**: 150.

ROBERTSON, W. R. B., 1930 Chromosome studies. V. Diploidy and persistent chromosome relations in partheno-produced Tettigidae (*Apotettix eurycephalus* and *Paratettix texanus*). J. Morph. and Physiol. **50**: 209–257.

SANSOME, F. W. and PHILP, J., 1932 Recent advances in plant genetics. Philadelphia: P. Blakiston's Son and Co., Inc.

STURTEVANT, A. H., 1923 Inheritance of direction of coiling in Limnaea. Science **58**: 269–270.

WHITING, P. W., 1932 Mutants in Habrobracon. Genetics **17**: 1–30.

ZELENY, CHARLES and FAUST, E. C., 1915. Size dimorphism in the spermatozoa from single testes. J. Exp. Zool. **18**: 187–240.

MUTANTS IN HABROBRACON. II[1]

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A general account of mutations in the parasitic wasp, *Habrobracon juglandis* (Ashmead) with descriptions of the mutant types has been published

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(P. W. WHITING, 1932). This included most of those found previous to April, 1931. The present paper deals with mutations which have come to light since that date, together with a few that occurred previously which had not been sufficiently studied to report.

THE MUTANT TYPES

Bar eyes. W. F. DUNNING (1931, p. 519) has reported a recessive mutant type which she calls bar eyes. Males were fertile but 121 tested females proved sterile (p. 523).

Kidney, k (eyes), and its allelomorphs small-eyes, k^s and extreme-small, k^e. A mutation to kidney, *k* (eyes) has been reported with a brief description of the mutant type (P. W. WHITING, 1932, pp. 20–21). When the wasps are bred at 30°C compound eyes and ocelli are reduced in size or absent (plate 1, figures 7–20) and the majority of specimens are inviable, many dying in cocoons as elongate pupae often with small heads (plate 5, figures 136–137). At lower temperatures kidney is highly viable and fertile in both sexes.

W. F. DUNNING (1931, p. 523) obtained two mutant types, "small and its allelomorph extreme small." These have proved allelomorphic with kidney. Small-eyes, k^s, (plate 1, figure 3) and extreme-small, k^e, (plate 1, figures 4–6) are of normal viability and fertility. A study has been made of dominance in the kidney-extreme-small compound females as affected by temperature differences (B. R. SPEICHER 1932).

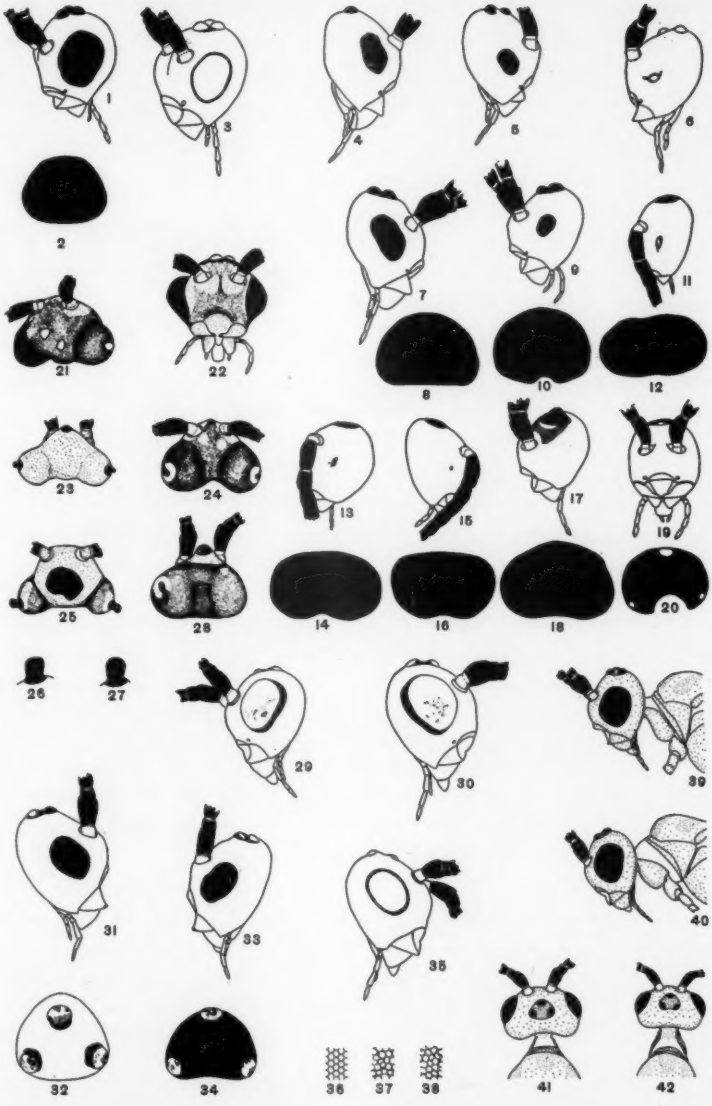
Eyeless, el. W. F. DUNNING (1931, p. 519) has reported a mutation to eyeless. Heads of the mutant type are much malformed when the wasps are bred at 30°C with large lobes on either side (plate 1, figures 21–25, 28). At lower temperatures these lobes fail to appear. Rudiments of compound eyes may be present (plate 1, figures 26–27). Wings tend to be somewhat wrinkled. Both sexes are fertile but females somewhat weak. Viability of males is about fifty percent that of their sibs.

White, wh (eyes), and its allelomorph carrot, wh^c. In June, 1931, LYSBETH HAMILTON BENKERT bred from a virgin heterozygous (*ta/sl*) female a fraternity of 51 males including one with white eyes and colorless ocelli. White is of normal viability and fertility. In combination with shot-vein *sv*, white constantly shows small red flecks scattered in the posterior and ventral part of the eyes. White shot-veins, *wh.sv*, are therefore known as variegated (plate 1, figures 29–30).

In March, 1932, mutant males with carrot eyes appeared in an experiment by KATHRYN A. GILMORE on production of impaternate females. Eye color closely resembles orange. The mutation occurred in the spermatogonia of a white stumpy male changing *wh* to wh^c and involving at least

LEGEND FOR PLATE 1

FIGURES 1, 3–7, 9, 11, 13, 15, 17, 19, 21–25, 28–31, 33, 35.—Magnification 33×.
FIGURES 2, 8, 10, 12, 14, 16, 18, 20, 26–27, 32, 34, 36–38.—Magnification 80×.
FIGURES 39–42.—Magnification 22×.
FIGURES 1–2.—Head and ocellar region of wild-type.
FIGURE 3.—Head of small-eye.
FIGURES 4–6.—Heads of extreme-small.
FIGURES 7–20.—Heads followed by ocellar regions from same specimen of kidney.
FIGURES 21–28.—Heads and rudimentary eyes of eyeless.
FIGURES 29–30.—Heads of variegated.
FIGURES 31–34.—Heads and ocellar regions of crescent.
FIGURE 35.—Head of pebbled.
FIGURE 36.—Facets of wild-type.
FIGURES 37–38.—Facets of pebbled.
FIGURES 39, 41.—Prothorax and adjoining regions of wild-type.
FIGURES 40, 42.—Prothorax and adjoining regions of extended-head.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five spermatozoa. Eyes of white-carrot compound females are pale yellowish in color. Carrot is of normal viability and fertility.

Crescent, cr (*ocelli and eyes*). In August, 1931, KATHRYN A. GILMORE bred from a heterozygous (*My/c.l.n*) virgin female a fraternity of 35 males including one (in vial d) with "crescent" ocelli and small eyes (plate 1, figures 31–34). Crescent is of normal viability and fertility in the male but females seem to have fertility slightly reduced.

Pebbled, pb (*eyes*). In March, 1932, MILTON F. STANCATI bred from an orange defective (stock 3) female (which had been mated to an X-rayed male) sixteen orange defective males (and no females). Five of the males had a new eye character, pebbled, with facets irregularly arranged and eyes somewhat smaller than normal (plate 1, figures 35–38). Pebbled males are rather small in size and difficult to mate. Pebbled females are weak or sterile. Viability of males is normal: heterozygous females have produced males: non-pebbled 197, pebbled 193.

Extended-head, Eh (*prothorax*). In July, 1931, NEITA C. BOSTIAN (1931) X-rayed (dosage about 3840 R units) a mated wild-type (stock 1) female. This female produced wild-type females 1, males 4 and one male with "extended-head." In this mutant type the membrane dorsal to the prosternum is much expanded so that the head is thrust forward (plate 1, figures 40, 42). When dead and dried the mutant type cannot be distinguished from normal. Heterozygous females show the character which may therefore be called dominant. Homozygous females have fertility somewhat reduced. Extended-head males have their viability reduced somewhat less than fifty percent as compared with their sibs.

Bent-wings, bw. In June, 1932, MILTON F. STANCATI bred from a virgin female ($+/k^e$) a fraternity of 18 males, five of which had bent-wings (plate 2, figures 43–45). Bent-wings males occur in fraternities from heterozygous mothers in about one-quarter the frequency of normal brothers. Bent-wings females are apparently unable to sting active caterpillars. When placed with paralyzed caterpillars a few may sting, feed and lay eggs but the eggs fail to hatch.

Gabled, gb (*wings*). In March, 1932, MILTON F. STANCATI bred from an orange defective (stock 3) female (which had been mated with an X-rayed male) wild-type females 3, inviable black-eyed (biparental) male pupae 3 and orange defective males 25. Seven of these azygous males had wings which sloped when folded like gables of a roof. The mutant type is thus easily recognized in the vials. The wings (plate 2, figures 46–47) have venation more reduced than in any other mutant type. The factor causes very poor viability: gabled males are less than ten percent as frequent as their normal sibs from heterozygous mothers. Gabled females have not been produced.

Flare, fl (*wings*). In February, 1932, MILTON F. STANCATI bred from a wild-type (stock 1) virgin female eleven males two of which had wings in which the costal margin flared forward distally (plate 2, figures 48–50). Flare overlaps with wild-type so that classification is uncertain. Flare females produce among their few offspring some which are phenotypically normal.

Indented, in (*wings*). In December, 1931, MILTON F. STANCATI X-rayed (dosage about 1500 R units) as a five-day larva an orange defective (stock 3) female which was subsequently mated to a wild-type (stock 1) male. An F_1 female produced 18 males of which five had the primary wings somewhat indented toward the tip. Indented frequently shows irregular thinning of the radius veins beyond the stigma in primaries and irregular narrowing of secondaries. Viability is normal and females are fertile.

Cut, ct (*wings*). In February, 1931, MAGNHILD TORVIK-GREB observed in a fraternity (from oo^i. *YY* female by wild-type male) of 16 females, 3 biparental males and 19 azygous males, that six of the azygous males had cut wings. Some of the sisters showed cut segregating in 1:1 ratio among their sons. The trait (plate 2, figures 57–64) is very variable but may be readily recognized. Viability and fertility are normal.

Strap, sr (*wings*). In January, 1932, RAYMOND J. GREB found in a fraternity of 28 females and 39 males from a heterozygous (*sv*/o^i. *st*) female mated with a brother (*sv*) that three of the males occurring in vial *c* and two in vial *f* had strap wings. The trait is very pronounced (plate 2, figures 65–71) and segregates in numbers equal to wild-type from heterozygous mothers.

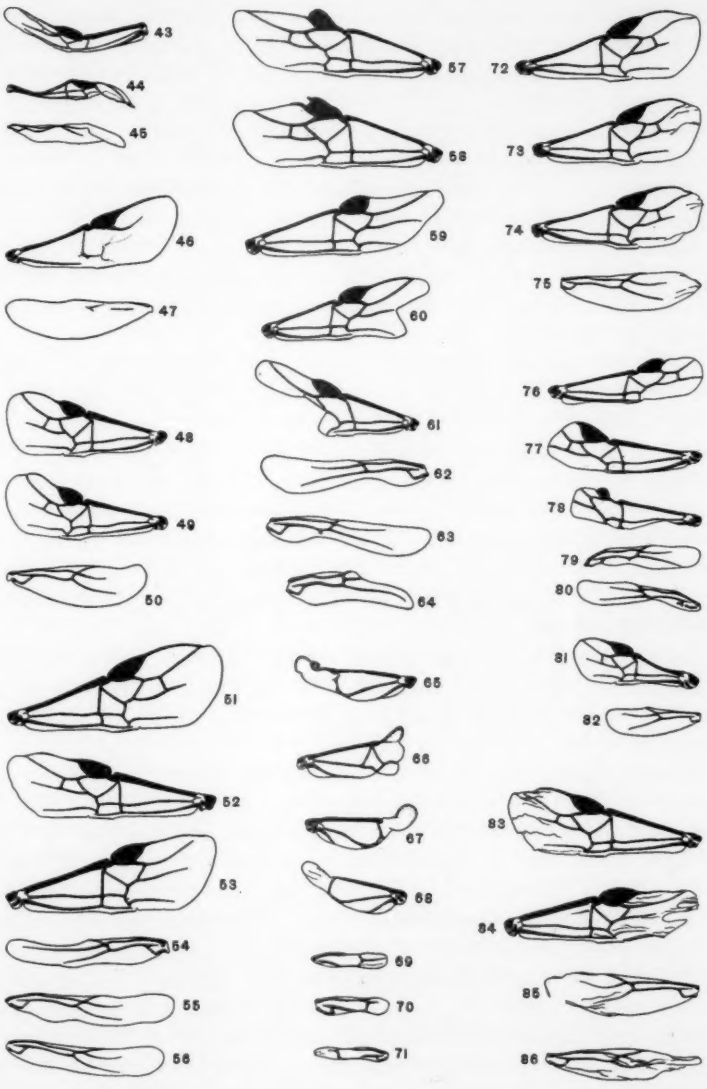
Pointed, p (*wings*). In July, 1931, NEITA C. BOSTIAN (1931) X-rayed (dosage about 3200 R units) a wild-type mated female. One of the daughters produced 19 wild-type females, 8 wild-type males and 12 males with pointed wings. The tips of the wings of this mutant type, both primaries and secondaries, are narrowed and wrinkled, the narrowing being especially noticeable in the radial cell. Females are sterile: they sting caterpillars, feed and lay eggs, living for several days, but the eggs fail to hatch. Pointed males appear in normal numbers from heterozygous mothers.

Truncated, td (*wings*). In December, 1931, MILTON F. STANCATI X-rayed (dosage about 2000 R units) as a five-day larva an orange defective (stock 3) female which was subsequently mated to a wild-type (stock 1) male. An F_1 female produced nine males (including two inviable pupae) of which five had truncated wings. The trait is variable (plate 2, figures 76–80) but easily recognized and does not overlap with wild-type as does truncate, *tr*, previously described (P. W. WHITING 1932). Truncated females are weak, failing to sting caterpillars or to oviposit. Truncated males appear in normal numbers from heterozygous mothers.

LEGEND FOR PLATE 2

Wings of various mutant types. All shown from dorsal side except figures 43–45, which are viewed from costal margin. Magnification 13×.

FIGURES 43–45.—Bent wings.
FIGURES 46–47.—Gabled.
FIGURES 48–50.—Flare.
FIGURES 51–56.—Indented.
FIGURES 57–64.—Cut.
FIGURES 65–71.—Strap.
FIGURES 72–75.—Pointed.
FIGURES 76–80.—Truncated.
FIGURES 81–82.—Small-wings.
FIGURES 83–86.—Crepe-wings.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Small-wings, sw. In March, 1933, MAGNHILD TORVIK-GREB bred a fraternity of fifteen males and several females (from $+/o \times n$). Six of the males showed a mutant trait, small-wings (plate 2, figures 81–82). Ratio of non-small-wings to small-wings were for vials a, b, c, respectively: 1 to 2; 1 to 2 and 7 to 2. Venation appears perfectly normal but wing size is greatly reduced and there is no overlapping with wild-type. Small-wings is of good fertility and viability.

Crepe-wings, cw. In April, 1932, MILTON F. STANCATI bred from a heterozygous (*o.d./+*) female (from stock 3 female by stock 1 male) a fraternity of seven males including two with wings irregularly wrinkled. The trait (plate 2, figures 83–86) is variable but easily recognized. The mutant type is somewhat over fifty percent viable as compared with sibs. Females are of decreased fertility.

Droopy, dr (wings). In March, 1933, KATHRYN A. GILMORE found a mutant type with wings held out and sloping downward (plate 4, figure 122). She bred from a virgin female heterozygous for tapering, *ta*, and crescent, *cr*, a fraternity of males as follows: wild-type 2, tapering 2, droopy 1, droopy-crescent 12, droopy-crescent-tapering 7, inviable (in cocoons) 8 and a mosaic (No. 670) with tapering antennae, wild-type eyes and left wings both droopy, right both normal. The excess of droopy in the fraternity indicates a lethal linked with the dominant allelomorph. Droopy, which is linked with crescent, evidently occurred in the chromosome with the factor crescent.

Droopy males occur in good numbers but are likely to die in cocoons. They are less viable when reared at 23°C than at 30°C. Droopy females try to sting the caterpillars but have produced no offspring except in one case when droopy males only appeared.

Extended-wings, ew. In July, 1932, MILTON F. STANCATI bred a fraternity consisting of nine males and two females. Five of the males had extended wings. The mother had one tested sister among whose eight male progeny there were no extended-wings. Extended-wings is easily distinguished when the wasps are active but if they are etherized there seems to be no observable difference from wild-type. They must therefore be separated in the active condition making counting tedious. The following description of the trait was made by KATHRYN A. GILMORE: The secondaries are extended laterally at an angle of 45 to 60 degrees to the horizontal and droop somewhat, both angles apparently being due to deformation at axilla. The primaries may be held almost normally or may be extended laterally at an angle approaching or equal to the secondaries (never more). The primaries are not held down like the secondaries, but in the normal plane as though the axilla of the primary is normal and their position is due simply to a defect in the secondaries. The abdomen is slightly elevated as

if for balance, especially in cases where both pairs of wings are widely spread.

Seven extended-wings females were set with paralyzed caterpillars but died after a few days without laying eggs. Of five females set with active caterpillars, none laid eggs and only one caterpillar was stung.

Extended wings segregates freely from ivory, white and cantaloup. Heterozygous virgin females, *wh. c/ew*, produced males,— +98, *c* 80, *wh* or *wh. c* 219, *ew* 66, *ew. c* 56, *ew. wh* or *ew. wh. c* 119.

Club, cl (*tarsi and wings*). In July, 1931, NEITA C. BOSTIAN (1931) X-rayed (dosage about 3270 R units) a mated wild-type (stock 1) female. This female produced a fraternity consisting of one wild-type female, eight wild-type males, and eight males with "club-feet." Terminal segments of tarsi are much malformed, fused and swollen (plate 3, figures 91–96) and wings show more or less abnormality with tendency to form extra veins in the radial cell (plate 3, figures 87–90). Club males are about fifty percent as frequent as their normal sibs. Females fail to oviposit even when caterpillars have been stung by another wasp.

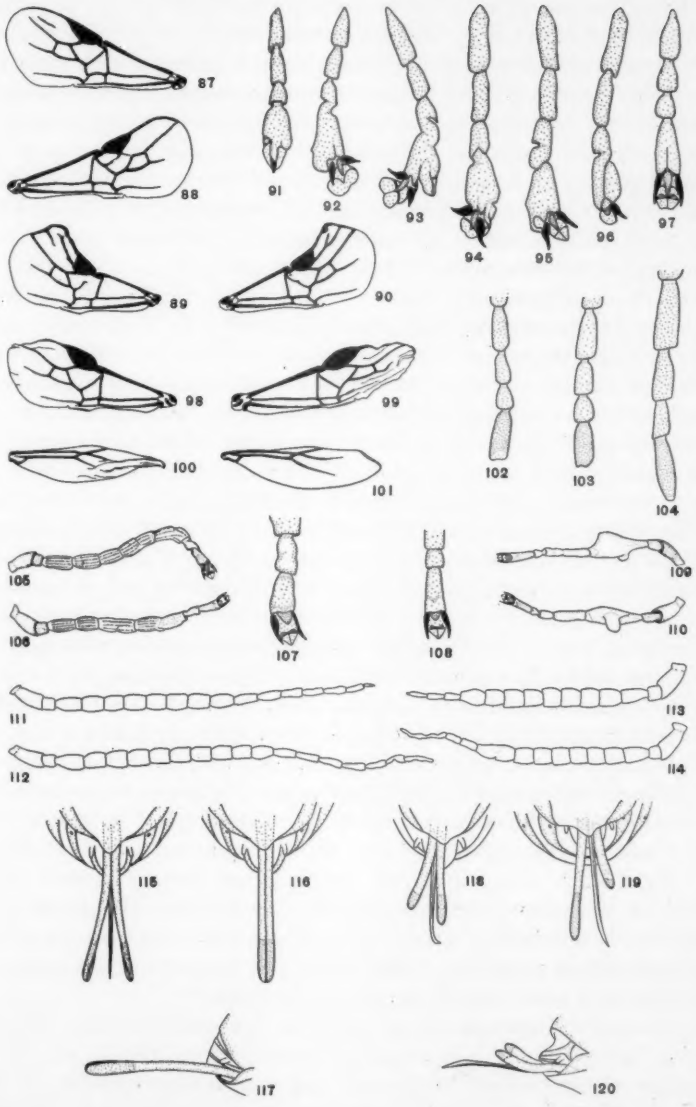
Footless, fo. In March, 1933, KATHRYN A. GILMORE bred from a heterozygous (*ta/o*) virgin female, a fraternity of 111 males among which 52 were "footless" through defect at the end of the fifth tarsal segment (plate 3, figures 102–104). Wings of footless tend to be more or less wrinkled (plate 3, figures 98–101). Footless males appear in good numbers from heterozygous mothers. The females drink honey, but cannot feed on caterpillars or lay eggs. The males mate readily.

Antennapedia, ap (*antennae*). Tapering, *ta*, antennae show considerable irregularity and fusion of segments, especially toward the tip. Drawings of some of these variations have been published (P. W. WHITING 1932, p. 9, plate 2). In the course of genetic work involving tapering, KATHRYN A. GILMORE observed tarsal-like claws on the terminal segments of the antennae of some of the specimens. Closer observation showed a more or less well-formed foot to be present (plate 3, figures 105–107). The distal segments may be modified to resemble a tarsus with the terminal segment provided with claws, arolium and calcanea as in the normal case. The foot may be present on one or both antennae and is often imperfectly developed. The trait occurs in a large but variable proportion of the individuals of a selected strain.

Leglike antennae. In April, 1933, RAYMOND J. GREB bred from a heterozygous (*sv. gy/wh. c. f*) female 81 males among which were 16 with leglike antennae. Distribution by vials through which the mother was successively passed was as follows for non-leglike and leglike respectively: vial a—14 to 0, vial b—26 to 0, vial c—17 to 2, vial d—6 to 12, vial e—2 to 2. Since each vial corresponds with four or five days of the mother's life, it is

LEGEND FOR PLATE 3

FIGURES 87–90, 98–101.—Magnification 13×.
FIGURES 91–97, 102–104, 107–108.—Magnification 80×.
FIGURES 105–106, 109–120.—Magnification 33×.
FIGURES 87–90.—Wings of club.
FIGURES 91–96.—Tarsi of club.
FIGURE 97.—Tarsus of wild-type.
FIGURES 98–101.—Wings of footless.
FIGURES 102–104.—Tarsi of footless.
FIGURES 105–107.—Antennae of antennapedia males.
FIGURES 108–110.—Antennae of leglike males.
FIGURES 111–112.—Antennae of aciform males.
FIGURES 113–114.—Antennae of aciform females.
FIGURES 115–117.—Tip of abdomen of wild-type females showing gonapophyses.
FIGURES 118–120.—Tip of abdomen of aciform females.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probable that no leglike were produced during the first two weeks after eclosion. The mutation to leglike doubtless occurred during the setting aside of the primitive germ cells, and mutant tissue probably composed the anterior part of both ovaries. Leglike could not be perpetuated because the males would not mate and no sisters were produced. Leglike is a much more extreme deviation than antennapedia. The entire antenna (plate 3, figures 109–110) except for the two basal segments has been changed so that the grooves for the sensillae are absent and yellow color similar to that of the legs has replaced the black. Fusion of several segments distal to the two basal and swellings on this mass suggest the malformed tibiae of certain types of inviable pupae (plate 5, figures 132–133). The foot (plate 3, figure 108) is provided with tarsal claws, arolium and calcanea similar to a foot from the normal position (plate 3, figure 97).

Aciform, ac (*antennae and female genitalia*). In December, 1932, MAGNHILD TORVIK-GREB bred a fraternity of three males from a wild-type (stock 1) female. Two of the males had antennae with segments of terminal half much reduced in diameter. The mutant type males are of normal viability. Gonopophyses of females are much shortened (plate 3, figures 118–120) and the sting is usually defective so that the females are sterile, failing to sting and feed on active caterpillars. Of ten females set with paralyzed caterpillars only one produced offspring, (two females and three males in vial a) then died in vial b. None lived over four or five days. The antennal character is easily distinguished, the basal nine or ten segments being normal, but terminal segments much narrower with more or less fusion and deletion in both sexes (plate 3, figures 111–114).

Dwindling, dw (*antennae*). In July, 1931, NEITA C. BOSTIAN (1931) X-rayed (dosage about 3200 R units) a mated wild-type female (stock 1). This female produced among her three sons, one with dwindling antennae. Dwindling shows much irregularity and fusion of antennal segments in the male, but proximal half of antenna is not affected (plate 4, figures 128–131). Females of dwindling cannot be distinguished from wild-type phenotypically. Many daughters from heterozygous females crossed with dwindling males have been set. All that have bred have produced both wild-type, and dwindling males, but many have proved sterile, probably the homozygous dwindling. Dwindling males are numerically equal to somewhat over two-thirds of their normal brothers.

Attenuated, at (*antennae and genitalia*). In May, 1932, a fraternity was bred by ANNA R. WHITING consisting of males, fifty percent of which had antennae with much malformation and fusion terminally. Genitalia were also abnormal and the type could therefore not be perpetuated. The mutation has been reported and the mutant type figured (P. W. WHITING and ANNA R. WHITING). Antennae are similar in appearance to those of dwindling.

Glass, gl (*eyes, antennae and tarsi*). In October, 1931, LYSBETH HAMILTON BENKERT bred from a heterozygous (*o/ma*) virgin female a fraternity of thirty males including thirteen with "glass" eyes. Tests of seven sisters of the mother showed no glass, making it improbable that the orange grandmother was heterozygous for the mutant factor. The mutation may have occurred in gametogenesis of the orange grandmother or of the maroon grandfather, involving at least one gamete. Glass eyes are smaller than normal, the ommatidia being imperfectly developed (plate 4, figures 123 and 124). Ocelli are apparently not affected. Antennae are very slender and of uniform diameter (plate 4, figures 126a and 127a). The tibial spur of the prothoracic legs is small while those of the mesothoracic and metathoracic legs are lacking. The foot is much malformed and reduced in size with claws very small or lacking (plate 4, figures 124a and 124b). Glass females are weak and of decreased fertility. Glass males are of good viability.

Gynoid, gy (*antennae, abdominal sclerites*). In April, 1932, RAYMOND J. GREB bred from a heterozygous (*sv/o*^{i}. *st*) female by a shot-veins brother a fraternity consisting of ten females and thirty males besides a male (in vial a) with antennae short, resembling those of a female. The character was associated with increased sclerotization of the abdomen especially in the anterior region. Since these traits resemble female characters and appear late in development, the factor causes weak male intersexuality. The mutant type, called gynoid, is highly fertile and viable in both sexes. Gynoid females are indistinguishable from normal except by breeding test.

Honey, ho (*body color*). In June, 1932, KATHRYN A. GILMORE set a wild-type female from stock that had reverted from tapering. There were produced wild-type females 6, tapering females 2, wild-type males 4, tapering males 1 and one non-tapering male (in vial c) with black pigment of antennae, wings and body very much reduced. This trait, called honey, is associated with good viability but females are of reduced fertility. It is readily distinguishable in all parts of the body where the integument normally shows black pigment as in antennae, legs, sensory gonapophyses of the female, and in all parts of the wings.

Lemon, le (*body color*). In September, 1933, F. JANE MAXWELL found one male with "lemon" body color in a fraternity of 124 males bred from a virgin female. A wild-type (stock 25) female had been crossed with a cantaloup gynoid male which had been X-rayed (dosage about 3500 R units). The F_2 males bred from one F_1 virgin were wild-type 31, cantaloup 33, gynoid 31 (including the lemon mutant), and cantaloup gynoid 29. It seems unlikely that the mutation was caused by the treatment since in that case it should have occurred in fifty percent of the fraternity. The trait is very striking, the ground color being pale lemon yellow, rather than honey yellow as in wild-type and honey. The black pigmentation of the

LEGEND FOR PLATE 4

FIGURES 121–122.—Magnification 22×.

FIGURES 123, 125–131.—Magnification 33×.

FIGURES 124a–124b.—Magnification 50×.

FIGURES 121 and 122.—Thorax and wings of wild-type and of droopy respectively.

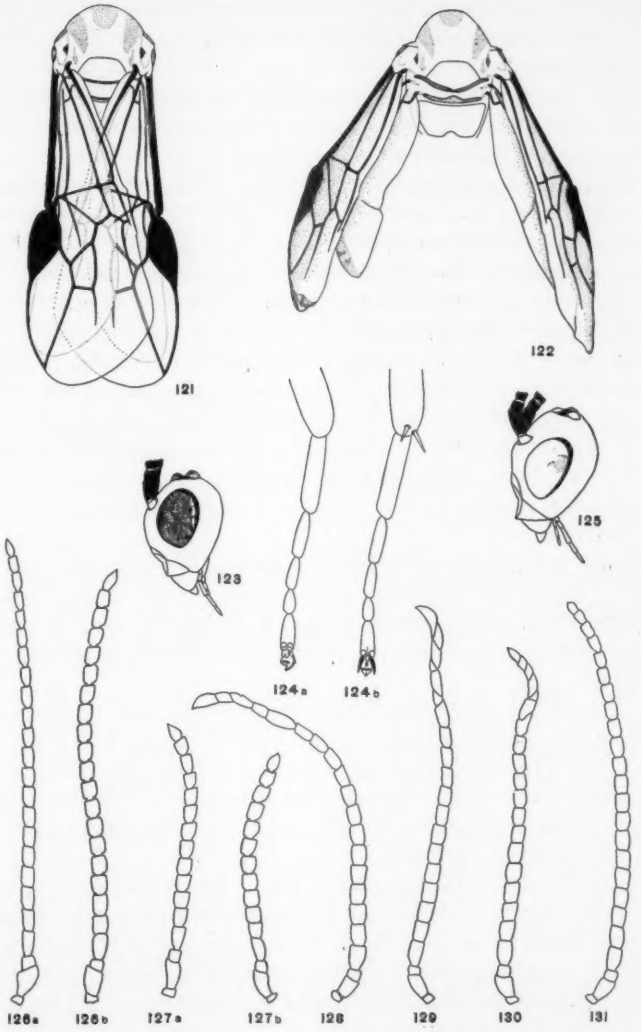
FIGURES 123 and 125.—Head of glass maroon and of glass cantaloup respectively.

FIGURES 124a and 124b.—Tarsus and end of tibia of right metathoracic leg of glass and of wild-type respectively.

FIGURES 126a and 126b.—Antenna of glass male and of wild-type male respectively.

FIGURES 127a and 127b.—Antenna of glass female and of wild-type female respectively.

FIGURES 128–131.—Antennae of dwindling males.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feet, female gonapophyses and wings appears perfectly normal while the antennae are black except for the two basal segments which are lemon yellow. Lemon thus shows a striking contrast to honey. Viability and fertility are normal.

Inviable pupae. The following examples will serve to illustrate some of the types of pupae differing in size and bodily proportions and dying on account of lethal or semilethal genetic factors.

Records of mutant type males segregating from heterozygous mothers not infrequently show more or less numerical deficiency either of wild-type or of the mutant type. When this is statistically significant it is taken to indicate presence of a linked lethal. Usually no record is made of inviable eggs or larvae but in some cases dead pupae are recorded. The first case of linkage in Habrobracon was found (P. W. WHITING 1921) between orange eyes and a factor having a lethal effect during the pupal stage. Crossovers were 19.5 percent and there were 142 pupae to 160 adults indicating that relatively few of the inviables died before pupation. No record was kept of eggs or larvae. The inviable pupae were of approximately normal proportions, perhaps slightly more slender, but they failed to spin cocoons and ranged in length up to 2.3 mm as compared with 2.9 mm for normal.

Beaded, *b* (legs), is semilethal. The mutant type survive much better at 23°C than at 30°C. Even at the higher temperature the majority attain the pupal stage, but few emerge from their cocoons. Tibiae and femora are much shortened and swollen, the swelling of the tibiae being confined to the distal half.

In March, 1932, LYSBETH HAMILTON BENKERT bred from one female 50 males of expected type and 31 which failed to emerge from their cocoons (604 LHB). These inviable specimens (plate 5, figures 132, 133) had tibiae expanded distally at first suggesting legs of beaded. The malformation was flattened, however, in cross section and affected the tibiae alone rather than both tibiae and femora.

The factor miniature is semilethal and is linked with orange eye color. Fifty-three percent of the mutant type eclose as compared with their normal sibs, about ten percent die in the cocoons and the remainder before pupation. Inviable pupae of miniature can be identified as to eye-color so that straights and crossovers can be separated in linkage tests. Variation in size and stage of development is very great in miniature pupae and size ranges up to about one millimeter only in length (plate 5, figures 138–142).

As previously stated, kidney are for the most part inviable at 30°C, many dying in cocoons as elongate pupae or prepupae with small heads and no eyes. They may be almost as long as normal pupae (plate 5,

figures 136–137). Kidney like miniature show a considerable range in stage of development at the time of death.

In July 1931, NEITA C. BOSTIAN (1931) X-rayed (dosage about 5780 R units) a mated wild-type (stock 1) female. One of the daughters produced a fraternity consisting of 15 normal males and 21 pupae (539 NCB) which died in their cocoons in a very immature condition (plate 5, figures 134–135). They failed to shed the larval integument and were almost colorless except that their eyes were deeply pigmented. They averaged about 1.5 mm in length.

In 1924, ISABEL POTTER found (26 IP) inviable pupae which segregated in equal numbers from their normal brothers and failed to emerge from their cocoons. They were of normal diameter but were much shortened, not surpassing 2.2. mm in length. Appendages were of approximately normal form but protruded irregularly from the body.

RE-OCCURRENCES OF FUSED

Three independent mutations to fused, *f* (antennae, tarsi, wings) were reported (Whiting 1931, p. 17) besides a male (No. 507) (Whiting 1932, pp. 299, 302–303) which was sterile but in all probability a mutant mosaic since it showed on the right side the peculiar characteristics of fused in antenna, tarsi and wings.

In breeding for biparental males, MAGNHILD TORVIK-GREB mated an orange defective female (stock 3) with an ivory fused male. Among the offspring there occurred an orange defective (grade 8) male (No. 381) with antennae, wings and tarsi typical for fused (and no pigmental asymmetry). Mated with a stock 3 female it produced 34 orange daughters, nine of which were bred producing fused among their orange sons. (No records were taken for defective.) This case was probably a mutation to fused in oögenesis. The fact that the "step-father" was fused must be attributed to chance.

In February, 1932, KATHRYN A. GILMORE found in vial b besides the 103 expected progeny from a heterozygous (*My.ta/wh.st*) virgin female, a male (No. 553) with antennae typical for fused. Since the legs were stumpy and wings unexpanded, the traits characteristic for fused could not be determined in those structures.

In February, 1932, RAYMOND J. GREB found in vial a among the progeny of a heterozygous (*My.sv/o^{i}.st*) female, a male (No. 556) with antennae and legs typical for fused. Since wings were shot-veins the indentation characteristic for fused could not be determined.

CARROT, *wh^{c}*, AND RE-OCCURRENCE OF SHOT-VEINS, *sv*, AND OF IVORY, *o^{i}*

In an experiment to obtain impaternate females, KATHRYN A. GILMORE crossed Minnesota-yellow tapering females with white stumpy males. The

LEGEND FOR PLATE 5

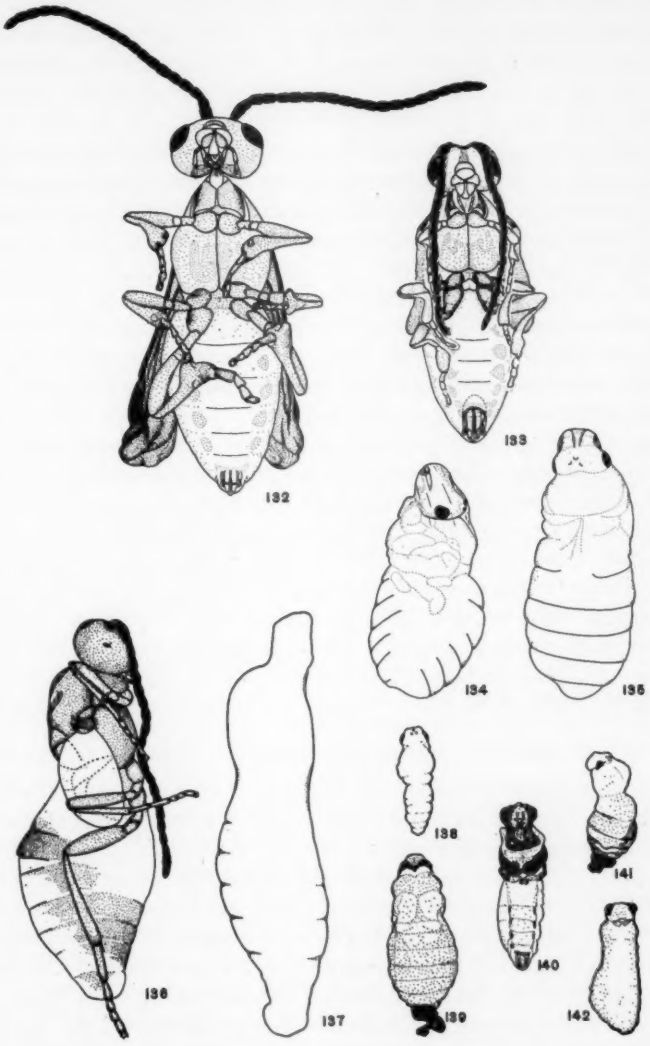
Inviable pupae and prepupae.—Magnification 25×.

FIGURES 132–133.—No. 604–LHB.

FIGURES 134–135.—No. 539-NCB.

FIGURES 136–137.—Kidney.

FIGURES 138–142.—Miniature.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F_1 females (*My.ta/wh.st*) were bred as virgins. Certain related cultures gave anomalous results including (1) apparent failure of linkage between white and stumpy, (2) shot-veins mutants similar to those which had previously been found in Minnesota-yellow stock, (3) lack of variegation in eyes of some of the white shot-veins wasps and (4) a new eye color later called carrot, wh^c.

Breeding test proved the new shot-veins to be in the same locus as the previous, *sv*. All F_1 females were shot-veins as were the 423 F_2 males bred. Pedigree analysis showed that the mutation occurred in the Minnesota-yellow tapering stock since two females of this stock were heterozygous for shot-veins.

Test of the white showed that ivory was also present. Ivory is not linked with stumpy and fails to show variegation in combination with shot-veins. Pedigree analysis showed that ivory was brought in by one of the white stumpy males and that this ivory was an independent mutation in the white stumpy stock.

Test of the new eye color showed it to be allelomorphic with white. The ivory stumpy white male transmitted ivory, stumpy and white to some (at least five) of his daughters, but to others (at least five) he transmitted ivory, stumpy and carrot. Carrot, wh^c, therefore occurred as a mutation from white in the spermatogonia of this male. Progeny of the five females (which were recorded only after the mutation was noticed) included 67 males which totaled (for stumpy and for eye color) as follows: wild-type 23, carrot 3, stumpy 1, carrot stumpy 12, "white" 13, "white" stumpy 15. Linkage between carrot and stumpy is ten percent as expected for white, while ivory, "white," shows independent segregation.

RELATION OF THE MUTATIONS TO X-RADIATION

In the case both of indented and of truncated the daughter of a female which was X-rayed and subsequently mated to an untreated male appeared to be heterozygous for the mutant factor. As regards pointed, inviable pupae 539NCB and possibly also dwindling, the mother was heterozygous and the daughter of a female that was mated and subsequently treated so that sperm as well as eggs were irradiated. Extended-head was produced (one mutant male among five) by a treated female. These mutations may therefore have been produced by the raying.

Certain mutations have appeared in X-ray experiments but the occurrence of the mutants was such that the mutations could not have been produced at the time of treatment. Thus the mother of club was heterozygous, but she herself as an adult was treated after having been mated. In the case of lemon there was produced only one mutant among 124 males. The grandfather had been X-rayed so that the mother should have

been heterozygous if the mutation had been produced at the time of treatment. As regards both pebbled and gabled the mother had been mated with a treated male. She was heterozygous for the mutant factor as shown by her azygous male progeny.

STAGE AT WHICH MUTATION OCCURS

If the mutants in a fraternity occur in numbers approximately equal to their brothers allowing for viability differences and if they are evenly scattered through the vials through which the mother was successively passed, then the mother may be assumed to be heterozygous for the new factor at least as regards her ovaries. Thus the mutant factor was present in one of her parents or the mutation occurred in her early development. If, however, there are only a few mutants or if the mutants occur in certain vials only, then it may be assumed that the mutation took place either in oögenesis or in one of the primitive germ cells of the female.

The number of mutant males among the total males of the fraternity are for the various mutant factors as follows:

Mother probably heterozygous: *pb* 5/16, *gb* 7/25, *in* 5/18, *td* 5/9, *p* 12/20, *cl* 8/16, *bw* 5/18, *fl* 2/11, *ct* 6/19, *cw* 2/7, *ew* 5/9, *gl* 13/30, *fo* 52/111, *sw* 6/15, inviable pupae 26IP 50 percent, inviable pupae 539NCB 21/36, inviable pupae 604LHB 31/81.

Mutation in oögenesis: *Eh* 1/5 (?), *sr* 5/39 (?), *le* 1/124, *wh* 1/51, *gy* 1/31, *cr* 1/35.

Uncertain cases due to small numbers: *dw* 1/3, *ho* 1/6, *ac* 2/3.

The occurrence of the sixteen mutants (among 81 males) with leglike antennae in vials *d* and *e* only (with the exception of two in vial *c*) indicates that the mutation occurred in one of the primitive germ cells of the mother.

The occurrence of 21 mutants (including one mosaic) to droopy (among 25 males) suggests that the mother was heterozygous but that a lethal was present linked with the dominant allelomorph.

The mother of the mutants to attenuated may have been heterozygous, although ratios from successive vials suggest that the mutation occurred in a primitive germ cell. Test of ten daughters of one white stumpy male showed that white, *wh*, was transmitted to five, but that a mutation to carrot, wh^c, had occurred in a spermatogonium or earlier and that this mutant factor was transmitted to the other five.

Of the seven mutations to fused, five appeared as single males, the mutation occurring in late oögenesis, one as a mosaic male, the mutation probably occurring in development, and one as offspring of a heterozygous female which may have received the factor from one parent.

In three cases the fraternities containing the mutants showed linkage of the mutant factor with previously known genes carried by the parents. Droopy showed linkage with crescent, attenuated with the dominant allelomorph to white and carrot with stumpy.

STERILITY OF TWISTED, *tw* (LEGS), FEMALES

Twisted, *tw* (legs), a mutant type previously reported (WHITING 1932, p. 16) has been bred by R. ELIZABETH CHALMERS in an endeavor to obtain a pure stock. Sixty-six females known to be heterozygous produced males, type 837 and twisted 448. Twenty of these which had been crossed with twisted males produced female offspring,—type 103, twisted 52, besides type and twisted males. Many twisted females were set but with little success. Six produced males,—type 50, twisted 24. Female offspring of two of these which had been mated with twisted males were type 28, twisted 4. One pairing of twisted by twisted gave all wild-type,—17 males and 11 females. One pairing of twisted by twisted gave males, type 30, twisted 2, and females, type 21, twisted 1. In no case when wild-type and twisted were both produced by any female was there a significant excess of twisted, the greatest being wild-type 5 and twisted 12.

These facts are consistent with the view that genotypic twisted is female sterile but that dominance is imperfect and that twisted phenotypes occur that are pure wild-type. Only one pairing of twisted by twisted gave all twisted, 15 males and 14 females, representing in all probability a fertile homozygous female by a twisted male.

SUMMARY

1. Thirty-two new viable mutant types in Habrobracon are reported in the present paper. Two, small-eyes and extreme-small, proved allelomorphic to kidney which has been previously reported. White and carrot (eye color) proved allelomorphic to each other.

2. Size or shape of eyes is affected by 7 of the mutant factors, antennae by 7, wings by 13, legs by 3, body color by 2, form of thorax by 1, abdominal sclerites by 1, male genitalia by 1 and female genitalia by 1.

3. All, except attenuated and leglike (?), are fertile in the male. Of the various types of females which have been adequately tested, 13 may be grouped as of normal or nearly normal fertility, 7 have fertility considerably reduced, while 8 are practically sterile. In two cases, club and footless, the failure to produce offspring is due to inability to feed on caterpillars and thus obtain nutriment for the development of eggs.

4. There are described seven different types of inviable pupae or prepupae of various shapes and sizes, each affected by a known genetic factor and differing in stage of development at which death occurs.

5. Of the four mutations to fused previously reported, two were in late oögenesis resulting in a single male in each case. In the third case the mother was heterozygous and in the fourth case the mutant was a single mosaic male. Three new re-occurrences of fused are here reported with a single male in each fraternity.

6. There are reported a re-occurrence of shot-veins in the stock in which the original mutation occurred and a re-occurrence of ivory which was masked for a time by white.

7. Six mutations may have been produced at the time of X-radiation, but four others appeared in X-ray experiments in such a relation that they could not have been produced by the treatment.

8. The new mutant types were produced by mothers probably heterozygous in 18 (or 19?) cases, while in 4 (or 5?) cases the mutation occurred late in oögenesis, the mutants being single males.

9. There is an indication that the mutation occurred in early germ cells in at least one case and in three cases the fraternity containing the mutants showed linkage of the new type with a previously known gene.

10. Breeding tests indicate that the previously reported mutant type twisted is almost female sterile and that wasps with twisted legs may be produced without the factor twisted.

ACKNOWLEDGMENTS

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LITERATURE CITED

BOSTIAN, NEITA CORRIHER, 1931 Sex ratios and mutants from X-rayed adult females of Habrobracon. Anat. Rec. **51**: 121.

DUNNING, W. F., 1931 A study of the effect of X-ray radiation on occurrence of abnormal individuals, mutation rate, viability and fertility of the parasitic wasp, *Habrobracon juglandis* (Ashmead). Genetics **16**: 505–531.

SPEICHER, B. R., 1932 Dominance of two kidney allelomorphs in *Habrobracon juglandis* (Ash.) Biol. Bull. **63**: 372–380.

WHITING, P. W., 1932 Mutants in Habrobracon. Genetics **17**: 1–30.

WHITING, P. W. and WHITING, ANNA R., A unique fraternity in Habrobracon. J. Genet. (in press).